BIOLOGICAL CRITERIA FOR STREAMS OF MISSOURI

A Final Report to the

Missouri Department of Natural Resources

from the

Missouri Cooperative Fish and Wildlife Research Unit

November 1997

DEVELOPMENT OF REGIONALLY BASED BIOLOGICAL CRITERIA FOR STREAMS OF MISSOURI

A report to the Missouri Department of Natural Resources from the Missouri Cooperative Fish and Wildlife Research Unit, 112 Stephens Hall, University of Missouri, Columbia, Missouri 65211

By
Charles F. Rabeni
Randy J. Sarver
Ning Wang
Gregory S. Wallace
Mark Weiland
James T. Peterson

November 1997

TABLE OF CONTENTS

ACKNOWLEDGMENTS

Funding for this project was provided by the U.S. Environmental Protection Agency through the Missouri Department of Natural Resources. This is a contribution of the Missouri Cooperative Fish and Wildlife Research Unit (Biological Resources Division, U.S. Geological Survey; Missouri Department of Conservation, University of Missouri-Columbia, and Wildlife Management Institute cooperating).

EXECUTIVE SUMMARY

The federal Clean Water Quality Act Amendments of 1972 codified the concept of "biological integrity" as the condition of an aquatic community inhabiting an unimpaired water body. The law profoundly affected water management by mandating that the condition of the aquatic life residing in streams and rivers be an endpoint to be measured. The perspective was changed from concentrating on what enters a stream or river, to the well being of the resident aquatic life. States were required to develop numerical or narrative biocriteria for their waterways to describe biological integrity. This project is an attempt to develop a systematic framework for biomonitoring streams of Missouri to describe and measure biological integrity.

The principle underlying the use of biocriteria to assess biological integrity is that unimpacted or least impacted streams have a fauna representative of the region and which is functioning in a natural manner. The process of biocriteria development involves determining biological attributes of communities in "reference conditions" that reflect integrity and then using these attributes as a standard to which all other sites and streams can be compared. We have followed the general guidelines of the U.S. EPA's Rapid Bioassessment Protocols. Benthic macroinvertebrates were chosen as sentinels of biological integrity because of their long history of use and their importance to the ecosystem.

Developing biocriteria involves several steps.

Step 1. *Ecoregion designations*

Ecoregions are geographical regions of the state with somewhat homogeneous environmental conditions and a homogeneous fauna. The aim in choosing ecoregions is to have a sufficient number so that the resident fauna has similarities, yet not have so many that the system becomes unmanageable. At least four statewide regionalization systems have been developed for Missouri and all have many elements in common. For our purposes we found that a good representation of the biota could be had with three ecoregions: the Ozark, Prairie, and Lowland. The fauna from streams within each region had good similarities, but were significantly different from fauna from other regions.

Step 2. *Selection of reference streams*

We started by reviewing the Missouri Water Atlas and MDNR maps which were used to identify perennial sections of all streams in the state. A list of candidate streams was developed based on watershed size and location entirely within an ecoregion. A step-wise process involving examination of human disturbance, stream size, stream channel morphology and condition, and migration barriers was then conducted with the advice of MDNR and Missouri Department of Conservation regional fisheries biologists. Of 92 candidate streams, 63 were field verified and rank ordered as to their suitability based on evidence of disturbance, riparian condition, heterogeneity of stream channel, abundance of large woody debris, aquatic vegetation, and normal color and odor. The 45 highest ranked streams were chosen.

Step 3. *Survey of the biota and habitat*

We developed standardized protocols for habitat analysis, and sampling and processing benthic invertebrates. All 43 reference streams were sampled in both the spring and fall of 1993.

Step 4. *Evaluating metrics for variability*

Using the reference streams dataset, we initially selected 14 metrics to be evaluated for their utility in describing biological integrity: Total number of taxa; Total number of Families; Number of Ephemeroptera, Trichoptera, and Plecoptera; the Biotic index (BI); Shannon's diversity index; Simpsons's diversity index; the percent that the dominant taxon is of the total number of individuals (% Dominant taxon); ratio of numbers of EPT to Chironomidae; ratio of Hydropsychidae to total Trichoptera; ratio of Shredders to total numbers; ratio of total Scrapers to total Filterers. An analysis of natural variation of metrics from reference streams indicated surprisingly low variation for Total taxa, Family, EPT, the BI, % Dominant taxon and both diversity indices. The ratio metrics were found to be too variable to be of much utility.

Step 5. *Evaluating metrics for sensitivity.*

Sensitivity, or the ability to detect degradation, was evaluated in a series of surveys comparing metrics from reference streams to metrics derived from streams with known degradations: including streams with poor water quality, poor habitat quality, and a combination of the two. We concluded that the metrics previously shown to have low variability also had the best sensitivity. Situations with poor water quality due to enrichment as measured by nutrient levels were readily detected by most metrics under most situations. Situations representing poor physical habitat conditions were less often detected by any metric. Situations representing the common occurrence of degraded physical habitat and poor water quality were detected most readily. Overall, sensitivity was much better in the Ozark region than in the Prairie region.

We also evaluated paired metrics. These are most often used to compare a reference to a test stream, where the similarity between the two invertebrate communities is quantified by, in our case, the Quantitative Similarity Index, the Percent Model Affinity, and the Coefficient of Community Loss and compared against a "threshold value." Indices were found to be good at detecting both habitat and water quality degradation and we recommend their use in situations where only a single reference stream is available or in upstream downstream evaluations.

Step 6. *Index Development*

Several metrics were shown to posses characteristic useful in biomonitoring. Many programs use individual metrics which is entirely appropriate. However, we have chosen to pursue the multimetric approach where several metrics, each providing somewhat different information about the invertebrate community, are combined into a single index—the Stream Condition Index. We selected four metrics—Total taxa, EPT, BI, and Shannon's Diversity Index—to be included in the SCI. The SCI was shown to have excellent discriminatory power for impaired streams, good discriminatory power for water quality degraded streams and little discriminatory power for habitat degraded streams. We offer criteria to rate streams as unimpaired, impaired, or highly impaired, based upon SCI values. These criteria have the potential to be adjusted to relate to Missouri's water quality standards.

ADDITIONAL CONSIDERATIONS

An efficient and sensitive biomonitoring protocol requires vigilance in reducing variation wherever possible—temporally, spatially, and in the laboratory. Many of our activities involved refinements and validation of our protocols.

We evaluated the adequacy of our field sampling because of concern that the common practice of taking a sample from a single site along a stream may be misleading. First we doubled the sampling effort by taking two sets of samples from the same site in several streams. Reproducibility was very good and we concluded that nothing would be gained by additional sampling at a single site. We then sampled a series of sites along several streams and evaluated the reduction in variation achieved by multiple site sampling. We concluded that, usually, sampling of one site was sufficient, while sampling two sites would be optimum. Sampling any more than two sites would probably not be worth the effort.

Because we sampled invertebrates from five different habitats at each site whenever possible, and analyzed them separately, we were able to conduct numerous evaluations of single vs. multiple habitat to address the question of how many and which habitats should be sampled. We determined that most metrics change significantly as the number of habitats at a site increases, and that most habitats had a distinct assemblage composition. We concluded that the only fair comparison between sites or groups of sites is the fauna from a single habitat or multiple habitats that are found at all sites. The question of which is superior, sampling a single habitat or sampling multihabitats, is less clear. Results were variable. In 1994, multihabitat data performed somewhat better than single, while for 1995, single habitat data was consistently, but not greatly, more sensitive. Because the sampling effort is relatively minor, perhaps multihabitat sampling in the field should be done, and decisions on which habitats to fully process could be determined by preliminary enumeration and analysis in the laboratory.

Reference conditions for a state-wide biomonitoring system need to be developed over a longer time period than the 3 years of this study. We found differences in metric values between spring and fall of the same year were not great, but differences from year to year were often significant. Until further temporal data is collected and evaluated, we recommend that reference sites be sampled each year that test sites are sampled. Our results show that a remarkably small subset of reference sites (perhaps 5-10) is all that is necessary to establish baseline conditions with low variation. The alternative is to average out metrics from reference sites over a period of years and use those scores to develop the SCI.

The identification of Chironomidae is a laborious process, which may account for half of all laboratory processing time. We evaluated the ability of our metrics to discriminate degraded situations using datasets with and without Chironomidae from the Ozark ecoregion. We concluded that the without-Chironomidae data showed identical or better results than the dataset containing Chironomidae. We conclude that the Chironomidae could be eliminated from the analyses with no loss of information when evaluating Ozark streams.

Using fish communities as descriptors of biological integrity showed promise. By sampling a minimum of seven reaches per stream with a back-pack electrofisher and evaluating with the fish Index of Biological Integrity, we were easily able to discriminate between impacted and unimpacted streams.

Chapter 1

INTRODUCTION

Evaluating the condition of water bodies by examining resident fauna has been well established in both theory and practice for several decades (Davis 1995). For example, the Saprobien system which listed indicator organisms associated with different zones of decomposition below sewage inflows and effectively evaluated organic degradation and downstream recovery was developed around the turn of the century (Kolkwitz and Marsson 1902). Biologists have doggedly pursued this topic since then, such that we now have a sophisticated understanding of the effects of anthropogenic actions on stream life, and numerous ways to measure it (Rosenberg and Resh 1993, Davis and Simon 1995). However, we have been more successful in gaining this knowledge than in putting it to use in a comprehensive and systematic way to preserve and protect aquatic resources.

What progress has been achieved in turning our knowledge into widely used management and regulatory techniques has been due primarily to federal legislation. Landmarks were the Federal Water Pollution Control Act in 1948, which formalized the process of water pollution control, and subsequent amendments to this law in 1965 (PL84-660) establishing the goal of "fishable and swimmable" waters, and in 1972 (PL-92-500) incorporating the concept of "biological integrity." Inclusion of the term biological integrity in the law had profound effects on water management because it mandated the ecological condition of the receiving waters as an endpoint to be measured.

A concerted effort was made in the early 1980s not only to produce an operational definition of biological integrity but also to codify ways to measure it. An effort led by the U.S. Environmental Protection Agency (EPA) provided the rationale for a program that used reference conditions within ecological regions which could then be used as a standard to measure test situations.

The most recent Clean Water Act amendment mandates that states work to develop narrative or numerical biocriteria. While progress has been modest, the concept of biological integrity has changed our perspective from one which concentrated on what enters a stream or river, to one focused on aquatic life. Development of useful biological criteria may be described as an exercise in reducing variation. Natural variation, i.e., attributes of the biotic community, occur because of geography (prairie vs. Ozarks), place in the watershed (headwater vs. mainstream), habitat within a stream section (pool vs. riffle), and seasonal life history processes. A second set of variants, biologist biases, is due to how the biota is sampled and analyzed. Reducing both natural and biologist induced variation allows a better chance to detect anthropogenic effects.

This project emphasized macroinvertebrates as the monitoring group—although some evaluations with fish were made. Benthic invertebrates are well known to be good monitors of stream quality and to act as integrators of a wide variety of physical, chemical, and hydrologic insults (Rosenberg and Resh 1993).

We used the EPA system as a basis (Plafkin et al. 1989) and the November 1997 Draft Revisions (see www.EPA.gov/owowwtr1/monitoring/AWPD/RBP) and modified it as needed for the particular conditions found in Missouri. Several distinct steps were involved in this process. We first classified the landscape in a hierarchical context: into ecological regions, or ecoregions, then by stream size within ecoregions, and finally by habitats within streams. Reference streams representing the best available conditions were selected and the resident biota was characterized. Community composition within and among regions was assessed by the ordination technique Detrended Correspondence Analysis (DCA). We evaluated commonly used indices or metrics for variability and redundancy. We then assessed metric sensitivity by comparing impaired streams to reference conditions. The best metrics were assembled into a stream condition index, and the stream index was used to develop biocriteria. Throughout the project we evaluated the adequacy of our field sampling methods and our laboratory procedures, the usefulness of multi- vs. single habitat sampling, and the need to always include Chironomidae. We recognize that this was one approach and that others could have been taken. Our intent was to offer a particular approach but to also follow the recent EPA revisions to Rapid Bioassessment Protocols for Use In Streams and Rivers which recommends the Performance-Based Methods System (PBMS) that stresses understanding, accuracy, and precision so data may be used in a number of ways. We would like nothing better than for the recommendations and conclusions of this project to be subjected to rigorous evaluation and validation and to be expanded, modified, or even supplanted with something more useful.

Chapter 2

AQUATIC ECOREGIONS OF MISSOURI

INTRODUCTION

Managing surface waters by developing biological criteria for the state of Missouri requires determining the environmental regions in which these surface waters can be expected to be similar. It may be useful at this point to define a region as a specific location that covers some extent of area and contains a certain degree of homogeneity of the characteristics used to define it (deBlij 1978). The term ecoregion was originally coined by Crowley (1967) and the first attempt to classify the system in mapped form was by Bailey (1976).

At least four statewide regionalization systems have been established for the State of Missouri. These systems, Watershed Provinces for Fisheries Management (Bauman 1945), Geologic Natural Features (Hebrank 1989), Aquatic System Classification System (Pflieger 1989), and Terrestrial Natural Areas (Thom and Wilson 1980) have been developed for specific purposes using different characteristics.

Recently the U.S. EPA developed an ecoregion system to assist managers of aquatic and terrestrial resources in understanding regional patterns of the realistically attainable quality of these resources. Ecoregions as defined by Omernik (1987) have been evaluated for streams and small rivers in Arkansas (Rohm et al. 1987), Ohio (Larsen et al. 1986, Whittier et al. 1987), Oregon (Whittier et al. 1988), Colorado (Gallant et al. 1989) and Wisconsin (Lyons 1989), and also for lakes in Minnesota (Heiskary et al. 1987). Ecoregion maps have been developed for the contiguous U.S. (Omernik 1987), and for each of the states mentioned above. Ecoregion maps are currently under development for portions of Mississippi and Alabama and for the State of Iowa. Maps for national, multistate, or individual states are available from the U.S. EPA, Environmental Research Laboratory, Corvalis, Oregon.

Of the several regionalization systems for Missouri, two approaches Omernik (1987) and Pflieger (1989) seem particularly appropriate for aquatic resource managers. No attempt has been made by this study to develop yet another, but because both aquatic regionalization systems have been proven to be useful, both were evaluated.

CLASSIFICATION SYSTEMS FOR MISSOURI

Aquatic Community Classification System (Pflieger 1989)

The Aquatic Faunal Region Map (Fig. 1) was reproduced for this study by the Geographic Resource Center, University of Missouri, Columbia. The original map (Pflieger 1989) was produced using the general composition of fish fauna and a few readily quantifiable physical attributes at 1608 localities to delimit the habitats of Missouri streams. Cluster analysis, coupled with a truncation procedure, was used to obtain a preliminary definition of habitat regions. A procedure called species composition analysis was developed to determine the species that characterize these regions, and to further refine the classification. Topographic patterning and the conformity of physical attributes to the locality groups defined by faunal analysis provided criteria for judging the plausibility of the classifications obtained. The classification system is meant to be applicable for classifying stream habitats in any area of Missouri, from which general collections of fishes or other elements of the stream biota are available for study (Pflieger et al. 1981).

Characteristics of Pflieger's Aquatic Community Classification System

The Aquatic Community Classification System (Pflieger 1989) divides Missouri into four principal regions, of which three (Ozark, Lowland, and Prairie) are of concern to this study. The fourth principal region (Big River) is recognized for the Missouri and Mississippi rivers. Characteristics of each region focus primarily on fish communities (Table 1). Fish communities are further subclassified by major drainage basins and by stream size.

Ecoregions of the Conterminous United States (Omernik 1987)

The Ecoregion map of Missouri (Fig. 1) was reproduced for this study by the Geographic Resource Center, University of Missouri, Columbia. The original map, Ecoregions of the South Central United States (Omernik and Gallant 1987), was produced as a supplement to Ecoregions of the Conterminous United States (Omernik 1987). One minor modification was made to the delineation of the Western Cornbelt Plains in response to a change in this ecoregion through a more detailed study in progress in Iowa.

Omernik's regionalization system is based upon a map overlay technique using maps of land use, land surface form, soils, potential natural vegetation, and other characteristic features important to each region along with qualitative analysis of the relative accuracy and level of generality of each map.

The qualitative approach to delineating ecoregions has the following advantages over a more quantitative approach (Gallant et al. 1989): 1) all available data (including spatial patterns of the variable itself), maps of characteristics that reflect regional variations and expert judgement can be incorporated; 2) the relative importance of particular environmental characteristics for influencing areal definition of a particular region commonly varies throughout the region; 3) even if relative importance of the environmental characteristics remained constant across a region, the quality of information portrayed on reference maps used for establishing the areal extent of the region often varies significantly, requiring continual modification of techniques. Reasons for this variation in quality result from the different source materials and base maps used to compile individual reference maps. Thus, the level of data generalization not only varies among different maps of the same scale, but within an individual map as well. This affects the accurate portrayal of information relative to its true geographic location, so it is necessary to manually adjust the placement of regional boundaries so as to avoid the "slivering" that would result from mechanically overlaying a set of maps; 4) because of inconsistencies mentioned in the previous two points, there is no way to preassess the decisions that will be required to draw regional boundaries. Preassessment is necessary for designing regionalization computer software; 5) the above reasons aside, the amount of computer storage required for all the digital information comprising the reference maps would be prohibitive.

Expert judgement is a critical part of the qualitative approach to regionalization. It allows for a review process by which some agreement can be met about regional boundaries. It is unlikely that two individuals developing regional boundaries, using a qualitative approach, would arrive at identical boundaries. However, when a strict quantitative approach is scrutinized it is also unlikely that independent investigators would delineate the same boundaries because of qualitative judgements necessarily involved, such as choosing which reference data, weightings, and classification techniques to use.

A report by the Subcommittee of the Ecological Processes and Effects Committee stated that the Ecoregion concept is a defensible classification technique for large areas and is superior to the classification methods currently used by most environmental managers. However, the lack of quantitative methods for testing regions and limited

Table 1. Fish Communities of the Principal Faunal Regions of Missouri (Pflieger 1989)

Lowland Faunal Region- Flowing Water	Common species- gizzard shad, longear sunfish, spotted sunfish, carp, orange spotted sunfish, bluegill, spotted bass, channel catfish, largemouth bass, shadow bass, blacktail shiner, bullhead minnow, mosquitofish, weed shiner, ribbon shiner, blackspotted topminnow, bluntnose minnow, emerald shiner, eastern redfin shiner, blackstripe topminnow, cypress darter, slough darter, bluntnose darter, tadpole madtom, dusky darter, blackside darter, and scaly sand darter
Ozark Faunal Region	Restricted species- chain pickerel, river redhorse, rock bass, Ozark bass, redear sunfish, largescale stoneroller, silverjaw minnow, bigeye chub, redspot chub, bluntface shiner, cardinal shiner, whitetail shiner, wedgespot shiner, Ozark minnow, Ozark shiner, duskystripe shiner, telescope shiner, spotfin shiner, steelcolor shiner, bleeding shiner, southern redbelly dace, eastern slim minnow, creek chub, northern studfish, plains topminnow, northern brook lamprey, southern brook lamprey, least brook lamprey, American brook lamprey, streamline chub, Ozark madtom, mountain madtom, checkered madtom, Neosho madtom, greenside darter, rainbow darter, White River darter, Current River saddled darter, barred fantail darter, golden fantail darter, yoke darter, least darter, Niangua darter, stippled darter, Current River orangethroat darter, Missouri saddled darter, banded darter, bluestripe darter, gilt darter, longnose darter, stargazing darter, mottled sculpin, Ozark sculpin and banded sculpin
	Common species- northern hogsucker, black redhorse, shadow bass, smallmouth bass, hornyhead chub, bigeye shiner, striped shiner, rosyface shiner, gravel chub, slender madtom and striped fantail darter
Prairie Faunal Region	Restricted species- mud minnow, brassy minnow, common shiner, bigmouth shiner, Topeka shiner, fathead minnow, plains killifish, trout-perch and plains orangethroat darter
	Common species- common carp, river carpsucker, quillback, white sucker, black bullhead, orangespotted sunfish, red shiner, sand shiner, western redfin shiner, creek chub, suckermouth minnow and johnny darter

guidance requires a relatively high level of expertise to produce defensible and reproducible subdivisions within state areas (U.S. EPA 1991).

Characteristics of Omernik's Ecoregions

The five ecoregions delineated in Missouri by Omernik are Interior River Lowlands, Ozark Highlands, Central Irregular Plains, Western Cornbelt Plains, and Mississippi Alluvial Plains. The four types of characteristics listed for each ecoregion can be found in Table 2.

Evaluation of Omernik's Ecoregions and Pflieger's Aquatic Community Classification System

When examining both regionalization systems it becomes obvious that the goals of the authors are in basic agreement. In fact the two systems are not exclusive of each other, but are merely different ways of explaining the same concept of homogeneity. Pflieger's Classification System takes an inductive theoretical approach using specific data to arrive at a general conclusion, while Omernik's Ecoregions takes a deductive theoretical approach in which general knowledge is used to predict a specific observation.

Maps of Pflieger's aquatic faunal areas and Omernik's Ecoregions can be overlain (Fig. 1) to show how well these two systems agree. The resulting map, Areas of Discrepancy between Omernik's Ecoregions and Pflieger's Aquatic Faunal Regions, shows that the total area of discrepancy amounts to approximately 18% of the state. In fact both authors realize that boundaries shown as lines are very commonly broad zones of transition. If areas of probable transition are removed the area of discrepancy decreases to approximately 12%. These areas consist of the northwest corner and the eastern edge of the state.

Further support for the close association between Omernik's Ecoregions and Ichthyogeographic regions was shown in an Oregon study (Hughes et al. 1987). Ichthyogeographic regions are aquatic ecoregions defined as large regions within which fish assemblages are expected to be relatively similar and among which fish assemblages are likely to be different. Because Pflieger's classification system is based upon fish community data it fits the definition of Ichthyogeographic Regions.

RECOMMENDATIONS

One of the goals of this study was to develop Ecoregions of Missouri as a basis for biocriteria. The evaluation of two regionalization systems shows close agreement. Data collected during this study supports the idea that a regionalized macroinvertebrate fauna exists, and that three ecoregions are sufficient to develop sensitive biocriteria. An additional evaluation of subregionalization of the data from this project is available from the lead author.

Table 2. Characteristics of Omernik Ecoregions.

Ecoregion	Land surface form	Potential natural vegetation	Land use	Soils
Interior River Lowlands	Irregular plains and open hills	Oak/hickory	Mosaic of cropland, pasture, woodland and forest	Alluvial and gray-brown Podizolic, wet Mollisols and Alfisols
Ozark Highlands	Open hills, high hills	Oak/hickoryoak/ hickory/pine	Mosaic of cropland, pasture, woodland and forest	Utisols
Central Irregular Plains	Irregular plains	Mosaic of bluestem prairie (bluestem, panic and Indian grass) and oak/hickory	Cropland, cropland with grazing cropland	Mollisols
Mississippi Alluvial Plains	Flat plains	Southern floodplain forest (oak, tupelo, bald cypress)	Cropland, cropland with grazing cropland, mosaic of cropland, pasture, woodland and forest swamp	Wet Inceptisols
Western Cornbelt Plains	Irregular plains	Bluestem prairie (bluestem, panic and Indian grass)	Cropland	Moist warm Mollisols (Udolls), Brunizems/ Humic Gley soils

Chapter 3

SELECTING REFERENCE STREAMS

INTRODUCTION

Development of biological criteria requires establishment of reference conditions. Reference conditions describe characteristics of waterbodies least impaired by anthropogenic activities and are used to define attainable habitat and biological conditions. Reference conditions are the standard by which impairment is judged.

Reference conditions can be established by identification of a number of sites that are positioned within each of the aquatic ecoregions, by evaluating an upstream-downstream situation where the reference is the upstream site, or by establishing paired streams or watersheds. For this project we emphasized identification of a number of sites within a region as partial development of scoring system, the Site Condition Index during 1993; however, we also evaluated the utility of the paired stream system in 1995.

To establish regional reference conditions, a set of streams of similar type and size are identified in each aquatic ecoregion. These sites must represent similar habitat types, be representative of the region, and exhibit biological integrity. Biological criteria can then be developed and used to assess impacted surface waters in the same region. Before reference conditions are established, regions of ecological similarity must be defined as addressed in Chapter 2.

METHOD FOR SELECTING REFERENCE STREAMS

A general method for selecting reference sites for streams and rivers has been described by Hughes et al. (1986). Ideally the reference site should be as little disturbed as possible and have characteristics that are representative of the region. These sites, if properly chosen, may serve as references for a large number of similar streams. It is important in the development of biological criteria to establish baseline conditions for the least impacted surface waters within each aquatic ecoregion. In many areas a return to pristine, or presettlement, conditions is impossible and goals for streams and rivers in extensively developed regions should reflect this.

A starting point was provided by the Missouri Water Atlas (MDNR 1986) and maps provided by the Missouri Department of Natural Resources (MDNR) which were used to identify the perennial sections of all streams and rivers in Missouri. Categories were then developed for those streams and rivers in which the drainage area of interest fell entirely within an ecoregion or in which the drainage area included substantial portions of two ecoregions. A list of all Missouri streams that were considered as candidates is provided as Table 1. In order to get the best representation from an ecoregion most reference conditions were to be selected from streams and rivers which were located entirely within an ecoregion.

The rationale for selecting the size of stream or river to be selected is attributed to the desire that conditions be "wadeable" and provide the best advantage for demonstrating ecoregion patterns. Although there is no agreement on the variety of ways to describe stream size (stream order, drainage area, miles to headwater, drainage area/unit discharge, etc.), there is some agreement that streams and rivers can be grouped into headwater, major tributary, and large river categories. Macroinvertebrate species richness and density have been demonstrated to be higher in major tributaries (Crunkilton and Duchrow 1991, Harrel and Dorris 1968, Minshall et al. 1985) and have a greater potential for showing spatial change. Predictable change in structure and function of stream ecosystems occurs along a longitudinal gradient from headwater to large river (Vannote et al. 1980, Wiley et al. 1990). The fact that major tributaries are often

Table 1. Perennial Streams of Missouri.

Name	Area mi²	Reference	Comments
	Western Cornbelt Plains		
Nishnabotna Atchison Co. 1:250,000 Neb. City		No	Extensive channelization
Rock Creek Atchison Co. 1:250,000 Neb. City		No	Perennial section in alluvium
Tarkio River Atchison Co. 1:250,000 Neb. City		No	Extensive channelization
Little Tarkio Creek Atchison Co. 1:250,000 Neb. City		No	Extensive channelization; oil wells on unnamed branch
Squaw Creek Holt Co. 1:250,000 Neb. City		No	Extensive channelization
Nodaway River Nodaway Co. 1:250,000 Neb. City		No	Extensive channelization; Clarinda, Bradyville, and College Springs STP's
102 River Nodaway Co. 1:250,000 Neb. City		No	Extensive channelization; 12 foot dam at Maryville
White Cloud Creek Nodaway Co. 1:250,000 Neb. City 1:100,000 Maryville 1:24,000 Bolckow NW	73.1	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Wildcat Creek Gentry Co. 1:250,000 Neb. City		No	Channelized below Stanberry; not perennial above Stanberry
Mill Creek Nodaway Co. 1:250,000 Neb. City		No	Extensive channelization; Elmo and College Springs influence
Long Branch Platte River Nodaway Co. 1:250,000 Neb. City 1:100,000 Maryville 1:24,000 Barnard	56.6	Yes	See file
Honey Creek Nodaway Co. 1:250,000 Neb. City 1:100,000 Maryville 1:24,000 Parnell W	88.6	Yes	See file
Western Cornbelt Plains/Central Irregular Plains			
Middle Fork Grand River Gentry/Worth Co. 1:250,000 Neb. City			Extensive channelization; Worth STP
East Fork Grand River Worth Co. 1:250,000 Neb. City 1:100,000 Maryville 1:24,000 Allendale	210.8	Yes	Kellerton IA. STP; See file
Grand River Gentry/Worth Co. 1:250,000 Neb. City		No	Channelized below Stanberry; not perennial above Stanberry

Table 1. Continued.

Name	Area mi^2	Reference	Comments
	Central Irregular Plains		
Grindstone Creek Dekalb Co. 1:250,000 Kansas City 1:100,000 St. Joseph 1:24,000 Weatherby	79.2	Yes	Maysville STP; See file
East Fork Big Creek Harrison Co. 1:250,000 Neb. City		No	Lamoni IA STP
West Fork Big Creek Harrison Co. 1:250,000 Neb. City/Centerville 1:100,000 Maryville 1:24,000 Bethany	148.5	Yes	See file
Sampson Creek Davies Co. 1:250,000 Neb. City		No	Extensive channelization; poor access
Weldon River Mercer Co. 1:250,000 Centerville		No	Extensive channelization
Little River Mercer Co. 1:250,000 Centerville		No	Extensive channelization
West Muddy Creek Mercer Co. 1:250,000 Centerville		No	Lake Paho influence
Thompson River Harrison Co. 1:250,000 Centerville		No	Extensive channelization
Big Muddy Creek Davies Co. 1:250,000 Moberly		No	Extensive channelization

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Marrowbone Creek Davies Co. 1:250,000 Moberly 1:100,000 Chillicothe 1:24,000 Nettleton	76.2	Yes	See file
Lick Fork Grand River Davies Co. 1:250,000 Moberly		No	Hamilton STP
Muddy Creek Mercer/Grundy Co. 1:250,000 Centerville		No	Extensive channelization
No Creek Livingston Co. 1:250,000 Centerville 1:100,000 Chillicothe 1:24,000 Farmersville	67	Yes	See file
West Locust Creek Sullivan Co. 1:250,000 Centerville 1:100,000 Trenton 1:24,000 Browning	104.5	Yes	See file
Locust Creek Sullivan Co. 1:250,000 Centerville 1:100,000 Trenton/Leon		Alt.	See file
East Locust Creek Sullivan Co. 1:250,000 Centerville		No	Milan STP; Milan reservoir
Sugar Creek Harrison Co. 1:250,000 Centerville		No	Small watershed; Lower reach channelized
Spring Creek Adair Co. 1:250,000 Centerville 1:100,000 Kirksville 1:24,000 Stahl	80.3	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Chariton River Adair Co. 1:250,000 Centerville		No	Extensive channelization; Lake Rathbun influence
Blackbird Creek Putnam/Adair Co. 1:250,000 Centerville		No	Unionville STP; Lake Thunderbird influence; mining
Mussel Fork Macon Co. 1:250,000 Moberly 1:100,000 Macon/Kirksville		Alt.	See file; Green Castle and Keytville STP's in upper watershed
East Yellow Creek Linn/Chariton Co. 1:250,000 Moberly		No	Extensive channelization; Marceline STP
West Yellow Creek Linn/Chariton Co. 1:250,000 Moberly		No	Brookfield STP
Big Creek Carroll Co. 1:250,000 Moberly		No	Extensive levies; probable channelization
Medicine Creek Putnam Co. 1:250,000 Centerville		No	Extensive channelization
Little Medicine Creek Mercer Co. 1:250,000 Centerville		No	Channelization; hog operation
Shoal Creek Caldwell Co. 1:250,000 Moberly 1:100,000 St, Joseph/Chillicothe		Alt.	See file
Fishing River Ray Co. 1:250,000 Kansas City		No	Metropolitan influence
Crooked River Ray Co. 1:250,000 Kansas City 1:100,000 Kansas City/St. Joseph		Alt.	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
East Fork Crooked River Ray Co. 1:250,000 Moberly 1:100,000 Marshall 1:24,000 Millville	74.7	Yes	See file
Wakenda Creek Carroll Co. 1:250,000 Moberly		No	Perennial section in alluvium
Turkey Creek Carroll Co. 1:250,000 Moberly		No	Perennial section in alluvium
Sniabar Creek Lafayette Co. 1:250,000 Kansas City		No	Metropolitan influence
Davies Creek Saline Co. 1:250,000 Jeff. City		No	Channelized; Higginsville reservoir influence
South Fork Blackwater River, Johnson Co. 1:250,000 Jeff. City		No	Extensive channelization
Post Oak Creek Johnson Co. 1:250,000 Jeff. City		No	Warrensburg STP; Metropolitan influence
Clear Creek Johnson Co. 1:250,000 Jeff. City		No	Whiteman AFB influence
Flat Creek Morgan Co. 1:250,000 Jeff. City		No	Sedalia SE STP; livestock
Haw Creek Morgan Co. 1:250,000 Jeff. City		No	Fish hatchery
Richland Creek Morgan Co. 1:250,000 Jeff. City		Alt.	Graveling

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Muddy Creek Pettis Co. 1:250,000 Jeff. City		No	Fish kills; livestock
Heaths Creek Pettis/Cooper Co. 1:250,000 Jeff. City		Alt.	No file
Petite Saline Creek Cooper Co. 1:250,000 Jeff. City 1:100,000 Jeff. City 1:24,000 Rocheport	199	Yes	See file
Lamine River Cooper Co. 1:250,000 Jeff. City		Alt.	Upstream of confluence with Flat Creek
Straight Fork Moreau River Moniteau Co. 1:250,000 Jeff. City		No	Tipton and Versailles STP's
Burris Fork Moniteau Co. 1:250,000 Jeff. City 1:100,000 Jeff. City 1:24,000 California S	66.5	Yes	See file
South Moreau River Miller Co. 1:250,000 Jeff. City		Alt.	Eldon STP
Bonne Femme Creek Boone Co. 1:250,000 Jeff. City		No	Atypical for ecoregion
Hinkson Creek Boone Co. 1:250,000 Jeff. City		No	Metropolitan influence
Perche Creek Boone Co. 1:250,000 Jeff. City		No	Mining
Bonne Femme Creek Howard Co. 1:250,000 Moberly		No	Mining

Table 1. Continued.

Name	Area mi²	Reference	Comments
Moniteau Creek Howard Co. 1:250,000 Moberly		No	Mining
South Grand River Cass Co. 1:250,000 Lawrence		No	Channelized; oil tank farm
Miami Creek Bates Co. 1:250,000 Lawrence		No	Butler STP; siltation
Little Dry Wood Creek Vernon Co. 1:250,000 Joplin 1:100,000 Nevada 1:24,000 Moundville	145.7	Yes	See file
Dry Wood Creek Vernon Co. 1:250,000 Joplin		No	Mining and acid drainage
Clear Creek Vernon/St. Clair Co. 1:250,000 Joplin 1:100,000 Nevada/Bolivar		Alt.	See file
North Fork Salt River Shelby Co. 1:250,000 Moberly		No	Extensive channelization
Middle Fork Salt River Monroe Co. 1:250,000 Moberly		No	Extensive channelization
West Fork Cuivre River Montgomery/Lincoln Co. 1:250,000 Jeff. City 1:100,000 Jeff. City		Alt.	Spans ecoregions
Moniteau Creek Moniteau Co. 1:250,000 Jeff. City 1:100,000 Jeff. City		Alt.	See file; California STP on East Brush Creek

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Central Irregular Plains/Ozark Highlands			
Horse Creek Cedar Co. 1:250,000 Joplin		No	Mining
Cedar Creek Cedar Co. 1:250,000 Springfield 1:100,000 Bolivar 1:24,000 Wagoner	112.2	Yes	See file
Brush Creek St. Clair Co. 1:250,000 Springfield		Alt.	Humansville STP
Turnback Creek Lawrence/Dade Co. 1:250,000 Springfield 1:100,000 Springfield		Alt.	See file; Billings and Greenfield STP's; Tank Farm at Lawrence
North Fork Spring River Jasper Co. 1:250,000 Joplin		No	Jasper and Lamar STP's
Center Creek Jasper Co. 1:250,000 Joplin		Alt.	At Carl Junction
Spring River Jasper Co. 1:250,000 Joplin		No	Syntex (dioxin); Verona STP
Shoal Creek Newton/Barry Co. 1:250,000 Tulsa		No	Chickens
Little Niangua River Hickory Co. 1:250,000 Springfield 1:100,000 Harry S. Truman Res. 1:24,000 Climax Springs	144.6	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Brush Creek Gasconade Co. 1:250,000 St. Louis		No	Cuba STP
Borbeuse River Gasconade Co. 1:250,000 St. Louis		No	Rolla, St. James, and Cuba STP's
	Ozark Highlands		
Bear Creek Cedar Co. 1:250,000 Springfield		Alt.	Fairplay STP; feedlots
Little Sac River Cedar Co. 1:250,000 Springfield		No	Springfield NW STP; Landfills; Fellows and McDaniels Lakes
Sac River Greene Co. 1:250,000 Springfield		No	Metropolitan influence
Pomme De Terre River Polk Co. 1:250,000 Springfield 1:100,000 Bolivar/ Springfield/Mountain Grove 1:24,000 Fair Grove	150.4	Yes	See file
Deer Creek Benton Co. 1:250,000 Springfield 1:100,000 Harry S. Truman Res. 1:24,000 Edwards	63.7	Yes	See file
Cole Camp Creek Benton Co. 1:250,000 Springfield		No	Cole Camp STP; graveling
Niangua River Dallas Co. 1:250,000 Springfield		Alt.	Marshfield STP
Barren Fork Miller Co. 1:250,000 Jeff. City		Alt.	NW Iberia

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Tavern Creek Miller Co. 1:250,000 Jeff. City 1:100,000 Lake Ozarks/Lebanon		Alt.	See file; Crocker STP
Little Maries River Maries Co. 1:250,000 Jeff. City 1:100,000 Lake Ozarks 1:24,000 Argyle	54.8	Yes	See file
Maries River Maries Co. 1:250,000 Jeff. City 1:100,000 Lake Ozarks/ Sullivan/Lebanon		Alt.	See file
Buffalo Creek McDonald Co. 1:250,000 Tulsa		No	Neosho STP; chickens
Indian Creek McDonald Co. 1:250,000 Tulsa		No	Chickens
Little Sugar Creek McDonald Co. 1:250,000 Tulsa		No	Bentonville AR influence
Big Sugar Creek McDonald Co. 1:250,000 Tulsa 1:100,000 Neosho 1:24,000 Powell	68.6	Yes	See file
James River Greene Co. 1:250,000 Springfield		Alt.	County road D, west of Turners
Flat Creek Barry Co. 1:250,000 Harrison		No	Cassville influence; Extensive grazing pressure
Roaring River Barry Co. 1:250,000 Harrison		No	Spring influence

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Crane Creek Stone Co. 1:250,000 Harrison		No	Crane STP and influence from Crane
Bull Creek Christian Co. 1:250,000 Harrison 1:100,000 Table Rock 1:24,000 Day	103.3	Yes	See file
Swan Creek Taney Co. 1:250,000 Harrison 1:100,000 Table Rock/ Ava/Springfield/ Mountain Grove		Alt.	See file
Beaver Creek Taney Co. 1:250,000 Harrison		No	Ava STP
Finley River Christian Co. 1:250,000 Springfield		No	Nixa and Ozark STPs
Bryant Creek Douglass Co. 1:250,000 Harrison		Alt.	Fish hatchery in upper watershed; losing stream
Hunter Creek Douglas Creek 1:250,000 Harrison		No	Trout Hatchery
Indian Creek Douglas Co. 1:250,000 Harrison		Alt.	Limited access
Spring Creek Douglas Co. 1:250,000 Harrison 1:100,000 Ava 1:24,000 Dora	63	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
North Fork River Douglas Co. 1:250,000 Harrison 1:100,000 Ava 1:24,000 Nichols Knob	181.7	Yes	See file
Spring Creek Ozark Co. 1:250,000 Harrison		No	Spring influence and trout hatchery
Warm Fork Spring River Oregon Co. 1:250,000 Poplar Bluff		Alt.	Cattle grazing
Eleven Point River Oregon Co. 1:250,000 Poplar Bluff 1:100,000 West Plains/ Spring Valley		No	Losing stream; graveling
Jacks Fork River Shannon Co. 1:250,000 Rolla 1:100,000 Spring Valley 1:24,000 Pine Crest	191	Yes	See file
Barren Creek Shannon Co. 1:250,000 Rolla		No	Losing stream; spring influence
Sinking Creek Shannon Co. 1:250,000 Rolla 1:100,000 Spring Valley 1:24,000 Round Spring	62.4	Yes	See file
Blair Creek Shannon Co. 1:250,000 Rolla 1:100,000 Spring Valley		Alt.	See file
Big Creek Shannon Co. 1:250,000 Rolla 1:100,00 Spring Valley 1:24,000 The Sinks	41.3	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Little Black River Ripley Co. 1:250,000 Poplar Bluff 1:100,000 Poplar Bluff 1:24,000 Flatwoods	99.6	Yes	See file
Fourche Creek Ripley Co. 1:250,000 Poplar Bluff		No	Fourche Lake; filamentous algae
Osage Fork Laclede Co. 1:250,000 Springfield		Alt.	At Dryknob; unpublished benthic data MDC
Beaver Creek Wright Co. 1:250,000 Springfield		No	High gravel bedload; cattle grazing and dairy
Whetstone Creek Wright Co. 1:250,000 Springfield		No	Mountain Grove STP; dairy
Current River Dent/Shannon Co. 1:250,000 Rolla		Alt.	At Cedar Grove
Wood Fork Wright Co. 1:250,000 Springfield		No	Dairy
Gasconade River Laclede Co. 1:250,000 Springfield		Alt.	At Competition
Spring Creek Pulaski Co. 1:250,000 Springfield		Alt.	At Spring Creek on county line
Big Piney River Texas Co. 1:250,000 Rolla		No	Cabool STP
West Piney Creek Texas Co. 1:250,000 Springfield 1:100,000 Mountain Grove 1:24,000 Bucyrus	76.8	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Little Piney Creek Phelps Co. 1:250,000 Rolla 1:100,000 Rolla 1:24,000 Yancy Mills	93.5	Yes	See file
Castor River Madison/Bollinger Co. 1:250,000 Rolla		No	Livestock related fishkills in 1992
Mill Creek Phelps Co. 1:250,000 Rolla		Alt.	Spring influence
Meremac River Crawford Co. 1:250,000 Rolla 1:100,000 Rolla 1:24,000 Cook Station	185.6	Yes	See file
Crooked Creek Crawford Co. 1:250,000 Rolla		No	Mine and smelter discharge; heavy grazing
Huzzah Creek Crawford Co. 1:250,000 Rolla 1:100,000 Rolla 1:24,000 Davisville	111.2	Yes	See file; minimal mine discharge
Courtois Creek Iron/Washington/ Crawford Co. 1:250,000 Rolla		No	Mine discharge
Hazel Creek Washington Co. 1:250,000 Rolla		No	All tributaries have barite tailings ponds which affect flow
Lost Creek Washington Co. 1:250,000 Rolla		Alt.	East of Berryman
Brazil Creek Washington/Crawford Co. 1:250,000 St. Louis 1:100,000 Rolla/Sullivan		No	Extensive clearing and limited access

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Indian Creek Franklin Co. 1:250,000 St. Louis		No	Mine tailings ponds; Pea Ridge Iron Mine; heavy gravel bedload
Big River Washington Co. 1:250,000 Rolla		No	Mining influence; Council Bluffs Lake
Logan Creek Reynolds Co. 1:250,000 Rolla		No	Mining influence
Cedar Creek Washington Co. 1:250,000 Rolla		No	Chickens
Mineral Fork Washington Co. 1:250,000 St. Louis		Alt.	Upstream highway 47
St. Francois Madison Co. 1:250,000 Rolla		No	Mining and tailings ponds
Marble Creek Iron/Madison Co. 1:250,000 Rolla 1:100,000 Piedmont 1:24,000 Des Arc NE	41.7	Yes	See file
Twelve Mile Creek Madison Co. 1:250,000 Rolla		Alt.	At Twelvemile; Cherokee Pass STP
Crane Pond Creek Iron Co. 1:250,000 Rolla		Alt.	At Brunot; Crane Lake influence
Big Creek Iron Co. 1:250,000 Rolla		No	Mining and smelter discharge
Clark Creek Wayne Co. 1:250,000 Rolla		No	Wappapello Lake influence

Table 1. Continued.

Name	Area mi^2	Reference	Comments
East Fork Black River Reynolds Co. 1:250,000 Rolla 1:100,000 Farmington 1:24,000 Johnson Shut-ins	57.4	Yes	See file; bedrock
West Fork Black River Reynolds Co. 1:250,000 Rolla		No	Lead mining
Sinking Creek Reynolds Co. 1:250,000 Rolla 1:100,000 Piedmont 1:24,000 Lesterville SE	66.8	Yes	See file
Middle Fork Black River Iron/Reynolds Co. 1:250,000 Rolla		No	Lead mining
	Interior River Lowlands		
Spencer Creek Ralls Co. 1:250,000 Quincy 1:100,000 Mexico/Quincy		Alt.	Curryville and Vandalia STPs
Peruque Creek St. Charles Co. 1:250,000 St. Louis		No	Metropolitan influence
Dardenne Creek St. Charles Co. 1:250,000 St. Louis		No	Metropolitan influence
Charette Creek Warren Co. 1:250,000 St. Louis		Alt.	East of Hopewell
Boeuf Creek Franklin Co. 1:250,000 St. Louis 1:100,000 Fulton 1:24,000 Dissen	97	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
St. John Creek Franklin Co. 1:250,000 St. Louis		Alt.	North of Clover Bottom
Joachim Creek Jefferson Co. 1:250,000 St. Louis		No	Lake, strip mine and metropolitan influence
Establishment Creek Ste. Genevieve Co. 1:250,000 St. Louis/ Rolla		No	Small lake; Bloomsdale STP; hogs
South Fork Saline Creek Perry Co. 1:250,000 Paducah		Alt.	West of Perryville
Cinque Hommes Perry Co. 1:250,000 Paducah		No	Perryville STP
Apple Creek Cape Girardeau Co. 1:250,000 Paducah 1:100,000 Carbondale 1:24,000 Friedheim	43.6	Yes	See file
Byrd Creek Cape Girardeau Co. 1:250,000 Paducah 1:100,000 Cape Girardeau/Carbondale		Alt.	See file
Ozark Highlands/Interior River Lowlands			
River Aux Vases Ste Genevieve Co. 1:250,000 Rolla 1:100,000 Farmington 1:24,000 Weingarten	47.8	Yes	Atypical geology for the Ozarks Ecoregion
Saline Creek Ste. Genevieve Co. 1:250,000 Rolla/Paducah 1:100,000 Farmington 1:24,000 Minnith	75	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Whitewater River Cape Girardeau Co. 1:250,000 Paducah		No	Past fishkills
Little Whitewater River Bollinger Co. 1:250,000 Paducah 1:100,000 Piedmont/ Cape Girardeau 1:24,000 Hurricane	31.3	Yes	See file
Bear Creek Wayne Co. 1:250,000 Rolla		Alt.	Northwest off Lowndes
Crooked Creek Bollinger Co. 1:250,000 Paducah		No	Lutesville STP
Little Saline Creek Ste. Genevieve Co. 1:250,000 Rolla/Paducah		Alt.	At highway N
Interior River Lowlands/Central Irregular Plains			
South Fabius River Marion Co. 1:250,000 Centerville 1:100,000 Quincy/Macon/ Kirksville/Keokuk		Alt.	See file; Edina STP
Middle Fabius River Lewis Co. 1:250,000 Centerville 1:100,000 Keokuk 1:24,000 Lewistown	348.4	Yes	See file; Lewistown STP
North Fabius River Schuyler/Scott Co. 1:250,000 Centerville		No	Channelized
Wyaconda River Clark Co. 1:250,000 Burlington		Alt.	Northwest of Benjamin

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Little Fox River Clark Co. 1:250,000 Burlington 1:100,000 Burlington/Keokuk		No	Most of watershed in Iowa
West Fork Cuivre River Lincoln Co. 1:250,000 Quincy		Alt.	At Montgomery Co. line
North Fork Cuivre River Lincoln Co. 1:250,000 Quincy		Alt.	At Davis, Briscoe or Silex
Bailey Creek Osage Co. 1:250,000 St. Louis		Alt.	North of Fredricksburg; SALT project
Cedar Creek Osage Co. 1:250,000 St. Louis		Alt.	Between Bonnots Mill and Frankenstein
Loutre River Montgomery Co. 1:250,000 St. Louis 1:100,000 Fulton 1:24,000 Montgomery City	196.8	Yes	See file
North River Marion Co. 1:250,000 Centerville 1:100,000 Quincy 1:24,000 Philadelphia	197	Yes	See file
Mississippi Alluvial Plains			
Cane Creek Butler Co. 1:250,000 Paducah 1:100,000 Poplar Bluff		Alt.	Crosses Ecoregions but is heavily influenced by lowlands
Huffstetter Lateral Stoddard Co. 1:250,000 Dyersburg TN/ KY/MO/IL 1:100,000 Sikeston 1:24,000 Bernie	Does not apply	Yes	See file

Table 1. Continued.

Name	Area mi^2	Reference	Comments
Little River New Madrid Co. 1:250,000 Dyersburg TN/ KY/MO/IL 1:100,000 Sikeston 1:24,000 Charter Oak		No	Recently dredged
Ash Slough Ditch New Madrid Co. 1:250,000 Dyersburg TN/ KY/MO/IL 1:100,000 Sikeston 1:24,000 Sikeston S	Does not apply	Yes	See file
Maple Slough Ditch Mississippi Co. 1:250;000 Dyersburg TN/ KY/MO/IL 1:100,000 Sikeston 1:24,000 East Prairie	Does not apply	Yes	See file

"wadeable," perennial, and best able to demonstrate ecoregion patterns is support for narrowing the focus of reference selection to streams of this general category.

Many candidate major tributary reference sites are not ecologically suitable and some process for selection must be used. The most objective method for selecting the type of reference sites needed in this study seems to be that of Hughes et al. (1986). The process involves six steps (Table 2), each of which should be discussed with knowledgeable resource managers and scientists who are familiar with the region. These professionals can also provide feedback during the selection process. Topographic maps and water quality professionals at the MDNR and Missouri Department of Conservation (MDC) and Fisheries Management Biologists at MDC were consulted during steps 1, 3, 4, and 5 of the reference stream selection process.

Field verification for access and determination of minimal disturbance was performed as part of the final selection process. Examples of indicators of good quality streams include: 1) extensive, old, natural riparian vegetation; 2) relatively high heterogeneity in channel width and depth; 3) abundant large woody debris, coarse bottom substrate, or extensive aquatic or overhanging vegetation; 4) relatively high or constant discharge; 5) relatively clear water with natural color and odor; 6) abundant diatom, insect, and fish assemblages; and 7) the presence of piscivorous birds and mammals.

Out of 92 candidate reference streams, 63 were field verified for minimal impact and the remainder were placed on the alternate list. Sixty-three streams were chosen for field verification based upon distribution and time and budget constraints. These streams were rank ordered, and 45 were chosen as the final reference streams with the remaining 18 being placed on the alternate list. Part of the rank ordering process included the comparison of drainage areas (step 2). Reference streams had drainage areas which differed by less than one order of magnitude, from 41 to 348 mi^2. Comparison of drainage area between ecoregions cannot reliably be done due to the karst geology and groundwater influence in the Ozark Ecoregion. This fact is supported by calculating the drainage area/mile of permanent stream ratio for streams across the State of Missouri (MDNR 1986).

Information concerning map references, drainage area in square miles, county, and comments is provided in Table 1 for each reference stream. Fig. 1 shows the distribution and gives map coordinates for each of the 45 reference streams. Table 3 provides more exact sampling locations on each stream.

Table 2. Steps in Determining Candidate Reference Streams and Rivers.

1. Human disturbance	Eliminate watersheds with concentrations of human, point source pollution, channelization or atypical diffuse sources of pollution (e.g. acidification, mine waste, overgrazing, clearcuts)
2. Stream size	Use watershed area and mean annual discharge instead of stream order (Hughes and Omernik 1983). Watershed areas and discharges of impacted and reference sites should differ by less than an order of magnitude.
3. Stream channel	Locate influent streams, springs and lakes; determine drainage pattern, stream gradient, and distance from major receiving water. Retain the stream type most typical of the region.
4. Locate refuges	Unless they result from local natural features atypical of the region, consider parks, monuments, wildlife refuges, natural areas, state and federal forests and grasslands and wilderness areas.
5. Determine migration barriers, historical connections among streams and known zoogeographic patterns.	Such information helps to form reasonable expectations of species presence and richness.
6. Suggest reference sites	Reject degraded or atypical watersheds and rank candidates by level of disturbance.

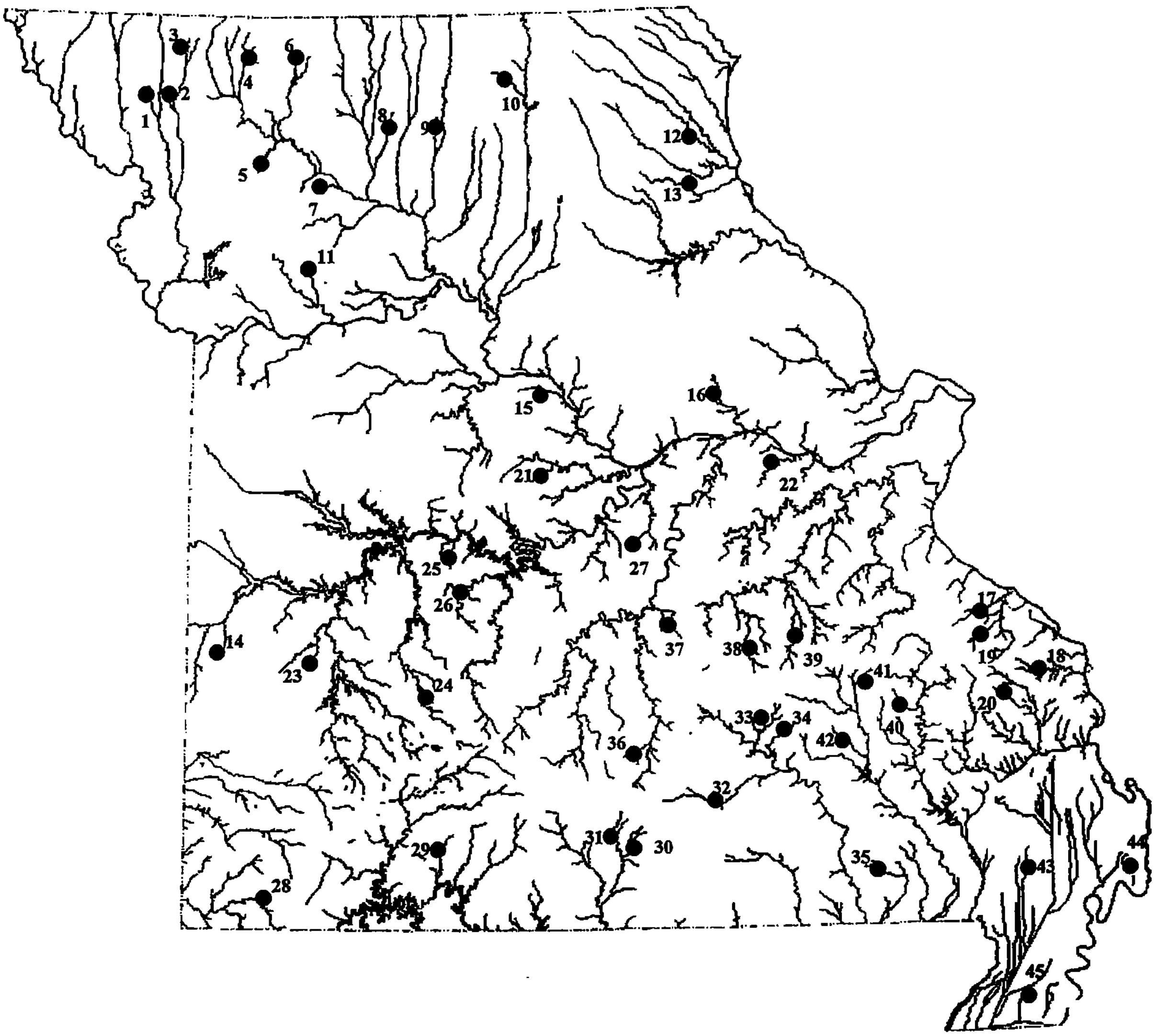


Fig. 1. Locations of all reference streams, 1993.

Table 3. Biological criteria project reference stream locations.

---

1. White Cloud Creek, Nodaway County—Section line 18 & 19; T62N; R35W; concrete bridge on county road.

2. Long Branch Platte River, Nodaway County—E1/2; Sec. 19; T62N; R34W.

3. Honey Creek, Nodaway County—Section line 13 & 24; T65N; R34W.

4. East Fork Grand River, Worth County—N1/2; Sec. 32; T66N; R30W; Highway 46 bridge.

5. Grindstone Creek, Dekalb County—NW1/4; Sec. 2; T58N; R30W; steel bridge on county road.

6. West Fork Big Creek, Harrison County—SW1/4; Sec. 22; T64N; R28W; steel bridge on county road.

7. Marrowbone Creek, Davies County—Section line 5 & 8; T58N; R27W; Highway HH bridge.

8. No Creek, Livingston County—Range line 24W & 23W; Highway 65 bridge.

9. West Locust Creek, Sullivan county—S1/2; Sec. 14; T61N; R21W; county road, bridge out but road still in fair condition.

10. Spring Creek, Adair County—N1/2; Sec. 24; T63N; R17W; steel bridge on county road.

11. East Fork Crooked River, Ray County—E1/2; Sec. 27; T53N; R27W; county road with steel bridge.

12. Middle Fabius River, Lewis County—NE1/4; Sec. 5; T61N; R8W; steel bridge on county road.

13. North River, Marion County—E1/2; Sec. 32; T58N; R7W; Highway Z bridge.

14. Little Dry Wood Creek, Vernon County—Section line 18 & 19; T35N; R31W; new concrete bridge on county road.

15. Petite Saline Creek, Cooper County—NE1/2; Sec. 13; T48N; R16W; newer concrete bridge on county road; enter from south in wet weather.

16. Loutre River, Montgomery County—N1/2; Sec. 28; T48N; R6W; at Graham Cave State Park.

17. River Aux Vases, Ste. Genevieve County—SE1/4; Sec. 27; T37N; R8E; concrete slab at low water ford.

18. Apple Creek, Cape Girardeau County—NW1/4; Sec. 4; T33N; R11E; concrete bridge on county road.

19. Saline Creek, Ste. Genevieve County—W1/2 Sec. 28; T36N; R9E; county road at Minnith.

Table 3 (continued).

---

20. Little Whitewater river, Bollinger County—N1/2; Sec. 1; T32N; R9E; concrete bridge on county road.

21. Burris Fork, Moniteau County—NW1/4; Sec. 5; T43N; R15W; concrete slab at low water ford.

22. Boeuf Creek, Franklin County—W1/2; Sec. 30; T44N; R3W; Hoeman road, concrete slab at low water ford.

23. Cedar Creek, Cedar County—N1/2; Sec. 9; T34N; R27W; steel bridge on county road.

24. Pomme De Terre River, Polk County—Section line 21 & 22; T32N; R21W; concrete slab at low water ford.

25. Deer Creek, Benton County—NE1/4 Sec. 31; T40N; R20W; at Haistain.

26. Little Niangua River, Hickory County—NW1/4; Sec. 2; T37N; R20W; concrete slab at low water ford.

27. Little Maries River, Maries County—W1/2; Sec. 34; T41N; R10W; concrete slab at low water ford.

28. Big Sugar Creek, McDonald County—N1/2; Sec. 21; T22N; R30W; Highway E bridge at Powell.

29. Bull Creek, Christian County—E1/2; Sec. 36; T25N; R21W; gravel low water ford.

30. Spring Creek, Douglas County—SW1/4; Sec. 23; T25N; R11W; concrete slab at low water ford.

31. North Fork River, Douglas County—Sec. 30; T26N; R11W; concrete slab at low water ford.

32. Jack's Fork River, Shannon County—Section line 31 & 32; T28N; R6W; Blue Springs Access.

33. Sinking Creek, Shannon County—Sec. 28; T31N; R4W; county road at end of Highway CC, concrete slab at low water ford.

34. Big Creek, Shannon County—NW1/4; Sec. 7; T30N; R3W; county road #250, concrete slab at low water ford.

35. Little Black river, Ripley County—N1/2; Sec. 25; T24N; R3E; end of Highway BB, gravel low water ford.

36. West Piney Creek, Texas County—NW1/4; Sec. 20; T30N; R10W; concrete slab at low water ford.

37. Little Piney Creek, Phelps County—SW1/4; Sec. 32; T36N; R8W; Lane Spring National Forest Service Campground.

Table 3 (continued).

38. Meremac River, Crawford County—SW1/4; Sec. 35; T36N; R5W; concrete slab at low water ford.

39. Huzzah Creek, Crawford County—S1/2; Sec. 20; T36N; R2W; at Red Bluff National Forest Service Campground.

40. Marble Creek, Madison County—S1/2; Sec. 18; T32N; R5E; Highway E at Marble Creek National Forest Service Campground.

41. East Fork Black River, Reynolds County—W1/2; Sec. 16; T33N; R2E; Johnson Shut-ins State Park.

42. Sinking Creek, Reynolds County—NE1/4; Sec. 20; T30N; R2E; concrete slab at low water ford.

43. Huffstetter Lateral Ditch, Stoddard County—Section corner 17, 18, 19, 20; T24N; R11E; county road bridge.

44. Ash Slough Ditch, New Madrid County—Township line 24N & 25N; R13E; Highway H bridge.

45. Maple Slough Ditch, Mississippi County—Township line 24N & 25N; R15E; county road bridge.

Chapter 4

HABITAT ASSESSMENT PROTOCOL

INTRODUCTION

Habitat assessment allows an understanding of the relation between habitat quality and biological conditions. Such assessments identify obvious constraints on the attainable potential of the site, assists in selection of appropriate sampling stations, and provides basic information for interpreting biological survey results (Barbour and Stribling 1991).

An important distinction must be made considering habitat analysis relations to the goals of this study. If the goal is evaluation of water quality only, then factoring out of the effects of physical habitat is important. However, if the goal is evaluating biological integrity, then habitat may be important to factor in as a cause.

Before a biological survey is conducted it is important to conduct a standardized habitat assessment. Because stream conditions vary considerably across an ecoregion, the investigator must make a decision whether the habitat quality of a study site is comparable to the habitat quality of a reference site. A conceptual relation between habitat quality and biological condition shown in Fig. 1 (Barbour and Stribling 1991) which demonstrates that the quality of the habitat can range from 0 to 100% of the reference, and can be categorized as nonsupporting, partially supporting, supporting, or comparable.

When the habitat quality of a study site is partially supporting to nonsupporting, compared to the reference site, the reduction in habitat quality may be all that is needed to judge impairment. Quantification of habitat quality may be as important as measuring the aquatic communities in the case of nonpoint source impacts. Guidance for this type of definitive assessment should be developed. In this study we expended considerable effort in determining how habitat degradation affects invertebrate communities.

ASSESSMENT

The basis for assessment of habitat quality lies in the derivation of a single numeric value through the process of totaling the scores from a number of habitat parameters. These habitat parameters are separated into three main categories: primary, secondary, and tertiary (Barbour and Stribling 1991).

Primary parameters are those that characterize the stream “microhabitats” and have the greatest direct influence on structure of the indigenous communities (Plafkin et al. 1989). Through field observation and measurement, parameters are scored from 0 (poor) to 20 (excellent). Secondary parameters measure the “macrohabitat” such as channel morphology characteristics. These parameters are scored from 0 (poor) to 15 (excellent). Tertiary parameters evaluate riparian and bank structure in the upstream section of the watershed. These parameters are scored from 0 (poor) to 10 (excellent). These three categories are weighted according to the influence upon the biological community, with primary parameters having more weight than secondary or tertiary characteristics.

A total score is obtained for each biological station and compared to a site–specific control or regional reference station. The ratio between the score for the study station and the score for the control or reference site provides a percent comparability measure. The study station is then classified on the basis of its similarity to expected conditions, and its apparent potential to support a similar community.

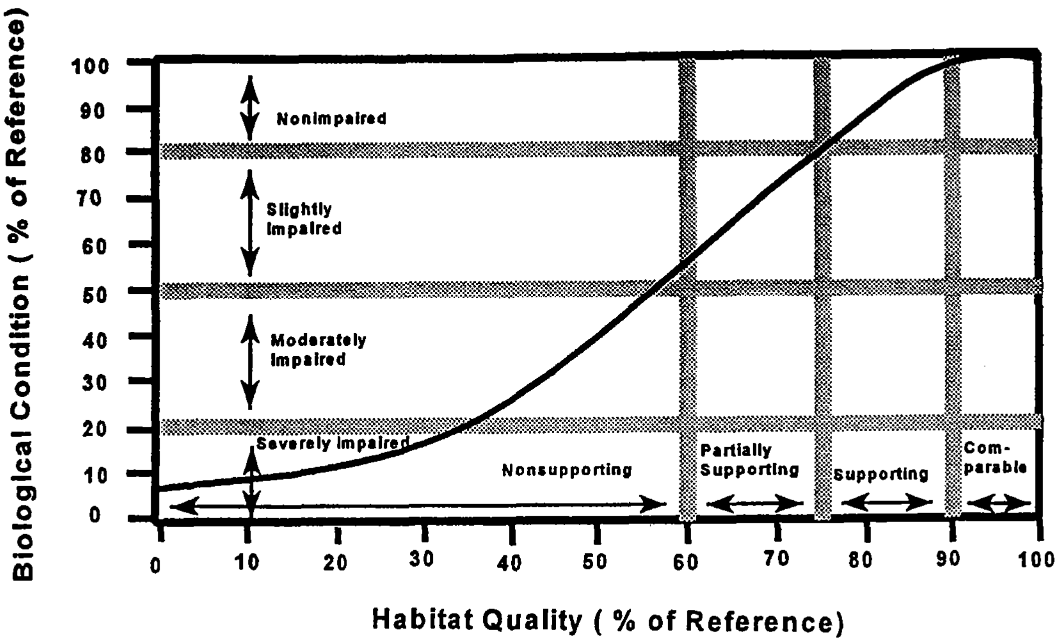


Fig. 1. Relation between habitat and the biota.

The assessment categories are as follows:

1) Comparable to Reference	≥90%
2) Supporting	75-89%
3) Partially Supporting	60-74%
4) Nonsupporting	<59%

This habitat assessment protocol uses the scoring matrix for Riffle/Run prevalence that was first described by Plafkin et al. (1989), and was later modified by Barbour and Stribling (1991) to contain more parameters. The assessment was also adapted by Barbour and Stribling (1991) to be used with Pool/Glide prevalence.

Riffle/Run Prevalence–Ozark Region

This format is appropriate for wadeable streams having a high gradient and a prevalence of riffles and runs. Further explanation of each parameter is provided in the following sections. Actual scoring should be recorded on the Riffle/Run Habitat Assessment Sheet (Appendix 1). All parameter scores should be agreed upon by team members.

Primary Parameters

These parameters are scored by selecting a reach of waterway that represents typical habitat. The evaluation is done in the immediate sampling area.

Bottom substrate/instream cover

This refers to availability of habitat for aquatic organisms. The presence of a broad variability in particle size of rock and gravel substrate is considered to be optimal for benthic macroinvertebrates. Instream materials such as logs, snags, tree roots, submerged and emergent vegetation, and undercut banks will provide habitat for a diversity of organisms. Habitat is evaluated by scoring predominant habitat types on a percentage basis.

Embeddedness

Embeddedness refers to how much of the surface area of larger substrate particles are surrounded by fine sediment or sand. Higher levels of sediment are thought to be correlated with lower biotic productivity. Two aspects of concern are: 1) the degree that the primary substrate is buried in fine substrate; and 2) the covering of the surface of the primary substrate with silt, sand, or organic floc. Both aspects will eliminate niche space and attachment area.

Stream flow and/or stream velocity

The size of the stream is known to influence the structure and function of aquatic communities. This parameter rates the quality of stream size with respect to: 1) the amount of water in small streams and 2) the variety of velocity-depth regimes in larger streams and rivers. The waterbody must be assigned into one of these categories before scoring.

Water quantity is most crucial for aquatic communities in small streams. Low flows $\leq$0.15 cms (5.0 cfs) will be more critical to the stream's ability to support aquatic communities.

In larger streams and rivers, i.e. flows >0.15 cms (5.0 cfs), velocity and depth is more important to maintenance of aquatic communities (Osborne and Herricks 1983, Oswood and Barber 1982). Four general categories of velocity and depth are optimal for benthic and fish communities: 1) slow (<0.3 m/s), shallow (<0.5 m); 2) slow (<0.3 m/s), deep (>0.5 m); 3) fast (>0.3 m/s), shallow (<0.5 m);and 4) fast (>0.3 m/s), deep (>0.5 m).

Habitat quality is reduced in the absence of one or more of these categories. Characteristics of water current make up the major factors of substrate quality and, by implication, the structure and composition of benthic communities (Minshall 1984).

Use of a flow meter. The U.S. Geological Survey is the Federal agency responsible for the national streamflow measurement program. The Survey has developed a number of guides for making flow measurements (Buchanan and Sommers 1969).

Flow (Q) is expressed as volume of water moving past a given stream cross section per unit of time. It is determined by multiplying the cross sectional area of water (A) in square feet times velocity (V) in feet per second, giving cubic feet per second. However, it is almost always necessary to break the channel into a number of sections because velocity varies greatly within the channel. At the left water edge and the right water edge the velocity is always zero except in the case of a vertical bank. Total flow is calculated by adding together the flow for each individual section.

The area for each individual section is calculated by using measurement tape readings as follows:

$$\frac{\text{Width measurement of following vertical} - \text{Width measurement of preceding vertical}}{2}$$

The number of subsections used in any flow measurement depends on the variability of velocities within the channel. Measurements are taken at all breaks in the gradient of the stream bottom and where any obvious changes in velocity occur. It is advisable to space the partial sections so that no partial section has more than 10% of the total flow contained within it. Equal widths of partial sections across the entire channel are not recommended unless the channel is extremely uniform. All data will be recorded on the Flow Measurement Data Sheet (Appendix 2).

Velocity variations with depth are accounted for by measuring flow at depths where velocity is equal to average velocity for the total depth. Proper measurement depths vary with water depth as follows: 1) if depth is less than 0.3 ft (0.1 m), measure at 0.5 of the depth; 2) if depth is from 0.3 to 2.5 ft (0.1–0.76 m), measure at 0.6 of the depth from the water surface; 3) If depth is greater than 2.5 ft (0.76 m), measure at 0.2 and 0.6 of the depth from the water surface and average.

Velocity is measured with a current meter attached to a rod or electronic current meter that provides a direct measurement. Operation and maintenance of current meters must be followed according to manufacturers' directions in order to assure reliable data.

Canopy cover

Canopy cover affects water temperature and energy availability for photosynthesis and primary production. A diversity of shading conditions is considered optimal.

Secondary Parameters

Channel morphology parameters are scored by using a standard reach of stream which is approximately equal to 20 mean stream widths. All scoring is done by visual estimation except for the measurement of stream depth and width.

Channel alteration

The formation of above water sediment bars is an indication of watershed erosion and allows a crude estimation of stream stability (Platts et al. 1983). Channelization involves a reduction in sinuosity and results in increased velocity and subsequent intensification of erosional effects (U.S. EPA 1983, Plafkin et al. 1989). Channel alteration also results in deposition, which may occur on the inside bends, below channel constrictions and where stream gradient flattens out (Plafkin et al. 1989).

Bottom scouring and deposition

The evaluation of bottom scouring and deposition is based upon an estimate of the percentage of substrate affected within the reach of interest. Characteristics to observe are scoured substrate and the degree of siltation in pools and riffles. Increases in velocity as a result of other channel altering factors are more likely to result in increased scouring and streambed erosion.

Riffle/width, or bend/width ratio

Hynes (1970) states that in an idealized system both riffles and meanders have a regularly occurring sequence which is related to stream width. Riffles repeat themselves on the order of 5–7 stream widths, and meanders are repeated at about 7–10 times the width. Since benthic communities rely upon substrate for shelter and food, it follows that any reduction in the natural sequencing may lower species diversity. These parameters assume that a stream with riffles or bends provides more diverse habitat than a straight run or uniform depth stream. Bends are included because low–gradient streams may not have riffles, but habitat can be produced by the amplified force of water at bends (Plafkin et al. 1989) resulting in well developed runs. The ratio is calculated by dividing the average distance between riffles or bends by the average stream width. If a stream contains riffles and meanders, use the feature that is dominant with the best habitat.

Lower bank channel capacity

Stream forms in Missouri vary from wide and shallow to narrow and deep, with heavily incised banks. The lower bank is the intermittently submerged portion of the stream cross-section from the normal high–water line to the lower water line. The lower channel defines the stream width. Rating is by observation of the width–to–depth ratio of the lower bank, and removal or distribution of riparian debris on the upper bank. The width-to-depth ratio is calculated by dividing the average top width of the *lower* bank by the height of the lower bank. This parameter is modified after Ball (1982) and is designed to evaluate the ability of the lower bank to contain normal peak flows.

Tertiary Parameters

Tertiary parameters focus upon the condition and form of riparian vegetation and bank stability of the upper bank. The upper bank is the land area from the break in the general slope of surrounding land to the normal high water line. The upper bank is normally vegetated and covered by water only during extreme high-water conditions.

Upper bank stability

This parameter is rated by observance of recent "bank sloughing" and the resultant movement of soil into the stream channel. The likelihood of erosion is usually increased with the steepness of the upper bank, since such banks often will not support vegetation (Ball 1982). Steep banks will evolve more readily from high velocity water compared to shallow banks where overflows are readily dissipated over the floodplain. Adjustments should be made in areas where clay composition, rip-rapping, or other human activities reduce erosion potential.

Bank vegetative stability (grazing pressure)

The primary concern of this parameter is the reduction of erosion from vegetative stability. Bank soil is generally held in place by plant root systems, although erosional protection may also be provided by boulder, cobble, or gravel material. Areas of higher vegetative cover receive higher ratings (Ball 1982, Plafkin et al. 1989). Vegetative stability is best rated in areas of little riparian zone disturbance. Areas exposed to grazing pressure or other disturbances should be evaluated under the second set of conditions (potential plant biomass) on the habitat assessment sheet.

Streamside cover

This measure rates the quality of nearstream riparian vegetation for its potential of fish refugia and nutrient input into the stream channel (Platts et al. 1983). A rating is obtained by visually determining the dominant vegetation type covering the exposed stream bottom, bank, and top of bank. Platts et al. (1983) found that streams bordered by shrub-sized vegetation produced higher fish standing crops than similar-sized streams bordered by trees; thus shrub dominance is rated as being optimal. In addition, leaf litter from the shrubs and other herbaceous plants is more rapidly available to instream communities than that from trees. The possibility that a fairly even mixture of shrubs and trees is more supportive of a diverse lotic biota is uncertain, but considered likely by some biologists (Ball 1982). Dominance by grasses and forbs is generally considered the least desirable stream cover.

Riparian vegetative zone width (least buffered side)

This parameter rates the entire riparian buffer zone on the side of the stream nearest to disruption (rowcrop, pasture, highway, surface mines, housing development, golf course, etc.). Decreasing buffer zone width is negatively correlated with shade (Lafferty 1987, Bartholow 1989), thus demonstrating its impact on water temperature, photosynthetic activity, and other temperature-dependant enzyme–mediated biological processes. Buffer strips can also slow runoff and filter organic material and sediment from entering the stream channel. The average width of the natural, undisturbed riparian vegetative zone is estimated for this parameter.

Glide/Pool Prevalence (Prairie and Lowland Streams)

All the parameters are essentially identical to those presented for assessment of riffle/run prevalent habitat, except for two parameters classified as primary. This habitat assessment would be used in Missouri when evaluating some low gradient streams such as those found in the southeast lowlands and prairie regions. Scoring should be recorded on the Glide/Pool Habitat Assessment Sheet (Appendix 3). All parameter scores should be agreed upon by team members.

Primary Parameters

Two primary parameters have been changed from the riffle/run prevalence to better evaluate low gradient streams.

Pool substrate characterization

Diversity in material composition of substrates has been discussed previously. For this parameter, pools with a diverse substrate are rated higher that those that are uniform.

Pool variability

This parameter rates the mixture of pool sizes within a stream reach. This variability is essential for the habitat to support a healthy fishery (Platts et al. 1983). Colonization by benthic communities is in response to available habitat. A variety of pool types and qualities will allow for a diversity of benthic macroinvertebrates, representing different sensitivities and preferences.

Physical Characterization/Water Quality

As part of the habitat assessment a Physical Characterization/Water Quality Data Sheet (Appendix 4) should be completed at all sites. Spaces for water quality, measurements of temperature, pH, dissolved oxygen, and conductivity are included on the data sheet.

Temperature

Normal temperature measurements may be made with any good quality mercury–filled Celsius thermometer. As a minimum, the thermometer should have a scale marked for every 0.1C. Make the readings with the thermometer immersed in water long enough to complete equilibration and report results to the nearest 0.1C.

pH

The pH value of a highly dilute solution represents hydrogen ion activity. Natural waters usually have pH values in the range of 4-9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals. The most accurate field measurement is done by potentiometric measurement using a glass electrode and reference electrode. Manufacturer's directions for use and maintenance of the pH meter must be followed.

Dissolved Oxygen

The ability of a body of water to support life is dependent on the level of dissolved oxygen (DO) contained within it. The level of DO in natural water depends on physical, chemical, and biochemical activities in the body of water. The minimum level of DO to support aquatic life is 5.0 mg/L for cool-warm waters (6.0 mg/L for cold waters). Accurate DO levels can be determined with relative ease through the use of a membrane electrode meter. Manufacturers' directions for maintenance and use of the meter should be followed.

Conductivity

Conductivity is a numerical expression of the ability of an aqueous solution to carry an electrical current. This ability depends upon the presence of ions, their total concentration, mobility, valence, relative concentrations, and on the temperature of measurement. Solutions of most inorganic acids, bases, and salts are relatively good conductors. Freshly distilled water has a conductivity of 0.5-2 umhos/cm. Conductivity of potable waters in the U.S. generally ranges from 50 to 1500 umhos/cm (Standard Methods for the Examination of Water and Wastewater 1980). Manufacturer's directions for use and maintenance of the selected conductivity meter must be followed.

CONCLUSION AND RECOMMENDATIONS

While the protocol outlined in this document is repeatable, and purports to evaluate a variety of potential stressors on the biota, its usefulness is limited. We found the relation between habitat and biological potential as theorized in Fig. 1 not to be accurate for Missouri streams. We will show later in this document that a substantial reduction in habitat quality is not reflected in any corresponding reduction in biological potential. There are two potential reasons for this: either the invertebrate communities are insensitive to habitat change—highly unlikely, or we are not yet measuring the correct variables. Further research is needed.

Appendix 1. Habitat assessment protocol riffle/run habitat assessment data sheet.

Date:	Analyst:	Station #:	Location:

Bottom substrate/instream cover[a]

Greater than 50% mix of rubble, gravel, submerged logs, undercut banks, or other stable habitat.	**(16-20)**	___
30-50% mix of rubble, gravel, or other stable habitat. Adequate habitat.	**(11-15)**	___
10-30% mix of rubble, gravel, or other stable habitat. Habitat availability less than desirable.	**(6-10)**	___
Less than 10% rubble, gravel, or other stable habitat. Lack of habitat is obvious.	**(0-5)**	___

Embeddedness[b]

Gravel, cobble, and boulder particles are between 0-25% surrounded by fine sediment or sand.	**(16-20)**	___
Gravel, cobble, and boulder particles are between 25-50% surrounded by fine sediment or sand.	**(11-15)**	___
Gravel, cobble, and boulder particles are between 50-75% surrounded by fine sediment or sand.	**(6-10)**	___
Gravel, cobble, and boulder particles are over 75% surrounded by fine sediment or sand.	**(0-5)**	___

Discharge [≤0.15 cms (5 cfs)] or Velocity/depth [>0.15 cms (5 cfs)]

If discharge 5 cfs or less:		
0.15 cms (5 cfs).	**(16-20)**	___
0.05-0.15 cms (2 -5 cfs)	**(11-15)**	___
0.03-0.05 cms (1-2 cfs)	**(6-10)**	___
<0.03 cms (1 cfs).	**(0-5)**	___
OR		
If discharge greater than 5 cfs:		
Slow (<0.3 m/s), deep (>0.5 m); slow, shallow (<0.5 m); fast (>0.3 m/s), deep; fast, shallow habitats.	**(16-20)**	___
Only three of the habitat categories present (missing riffles or runs receive lower score than missing pools).	**(11-15)**	___
Only 2 of the 4 habitat categories present (missing riffles or runs receive lower score).	**(6-10)**	___
Dominated by 1 velocity/depth category.	**(0-5)**	___

Canopy cover (shading)[c, d, g]

A mixture of conditions where some areas of water surface fully exposed to sunlight, and other receiving various degrees of filtered light.	**(16-20)**	___
Covered by sparse canopy; entire water surface receiving filtered light.	**(11-15)**	___

Appendix 1. (Continued).

Completely covered by dense canopy; water surface completely shaded OR nearly full sunlight reaching water surface. Shading limited to <3 hours per day.	**(6-10)**	___
Lack of canopy, full sunlight reaching water surface.	**(0-5)**	___

Channel alteration[a]

Little or no enlargement of islands or point bars, and/or no channelization.	**(12-15)**	___
Some new increase in bar formation, mostly from coarse gravel; and/or some channelization present.	**(8-11)**	___
Moderate deposition of new gravel, coarse sand on old and new bars; and/or embankments on both banks.	**(4-7)**	___
Heavy deposits of fine material, increased bar development; and/or extensive channelization.	**(0-3)**	___

Bottom scouring and deposition[a]

Less than 5% of the bottom affected by scouring and/or deposition.	**(12-15)**	___
5-30% affected. Scour at constrictions and where grades steepen. Some deposition in pools.	**(8-11)**	___
30-50% affected. Deposits and/or scour at obstructions, constrictions, and bends. Filling of pools prevalent.	**(4-7)**	___
More than 50% of the bottom changing frequently. Pools almost absent due to deposition. Only large rocks in riffle.	**(0-3)**	___

Riffle/width or bend/width ratio[a]

Ratio: 5-7. Variety of habitat. Repeat pattern of sequence relatively frequent.	**(12-15)**	___
Ratio: 7-15. Infrequent repeat pattern. Variety of macrohabitat less than optimal.	**(8-11)**	___
Ratio: 15-25. Occasional riffle or bend. Bottom contours provide some habitat.	**(4-7)**	___
Ratio >25. Essentially a straight stream. Generally all flat water or shallow riffle. Poor habitat.	**(0-3)**	___

Lower bank channel capacity[b]

Overbank (lower) flows rare. Lower bank W/D ratio <7. (Channel width divided by depth of lower bank.)	**(12-15)**	___
Overbank (lower) flows occasional. W/D ratio 8-15.	**(8-11)**	___
Overbank (lower) flows common. W/D ratio 15-25.	**(4-7)**	___
Peak flows not contained or contained through channelization. W/D ratio >25.	**(0-3)**	___

Upper bank stability[a]

Upper bank stable. No evidence of erosion or bank failure. Side slopes generally <30 degrees. Little potential for future problems.	**(9-10)**	___

Appendix 1. (Continued).

Moderately stable. Infrequent, small areas of erosion mostly healed over. Side slopes up to 40 degrees on one bank. Slight potential in extreme floods.	**(6-8)**	___
Moderately unstable. Moderate frequency and size of erosional areas. Side slopes up to 60 degrees on some banks. High erosion potential during extreme high flow.	**(3-5)**	___
Unstable. Many eroded areas. "Raw" areas frequent along straight sections and bends. Side slopes >60 degrees common.	**(0-2)**	___

Bank vegetation OR Grazing or other disruption[d]

Over 90% of the streambank surfaces covered by vegetation.	**(9-10)**	___
70-89% of the streambank surfaces covered by vegetation.	**(6-8)**	___
50-69% of the streambank surfaces covered by vegetation.	**(3-5)**	___
Less than 50% of the streambank surfaces covered by vegetation.	**(0-2)**	___
OR		
Vegetative disruption minimal or not evident. Almost all potential plant biomass at present stage of development remains.	**(9-10)**	___
Disruption evident but not affecting community vigor. Vegetative use is moderate, and at least one-half of the potential plant biomass remains.	**(6-8)**	___
Disruption obvious; some patches of bare soil or closely cropped vegetation present. Less than one-half of the potential plant biomass present.	**(3-5)**	___
Disruption of streambank vegetation is very high. Vegetation has been removed to 2 inches or less in average stubble height.	**(0-2)**	___

Streamside cover[b]

Dominant vegetation is mixture of tree and shrub.	**(9-10)**	___
Dominant vegetation is of tree form.	**(6-8)**	___
Dominant vegetation is grass or forbes.	**(3-5)**	___
Over 50% of the streambank has no vegetation and dominant material is soil, rock, bridge material, culverts, or mine tailings.	**(0-2)**	___

Riparian vegetative zone width (least buffered side)[e, f, g]

>18 meters.	**(9-10)**	___
Between 12 and 18 meters.	**(6-8)**	___
Between 6 and 12 meters.	**(3-5)**	___
<6 meters.	**(0-2)**	___

Totals ___

[a]From Ball 1982.
[b]From Platts et al. 1983.
[c]From EPA 1983.
[d]From Hamilton and Bergersen 1984.
[e]From Lafferty 1987.
[f]From Schueler 1987.
[g]From Bartholow 1989.

Appendix 2. Habitat assessment protocol flow measurement data sheet.

Date:	Analyst :	Station #:	Location:

Vertical	Tape Reading	Width of Vertical FV-PV/2	Depth	Area (A)	Velocity (V)	Flow (Q)
W_{lb}						
W_1						
W_2						
W_3						
W_4						
W_5						
W_6						

Appendix 3. Habitat assessment protocol glide/pool habitat assessment data sheet.

Date:	Analyst:	Station #:	Location:

Bottom substrate/instream cover[a]

Greater than 50% mix of rubble, gravel submerged logs, undercut banks, or other suitable habitat.	**(16-20)**	___
30-50% mix of rubble, gravel, or other stable habitat. Adequate habitat.	**(11-15)**	___
10-30% mix of rubble, gravel, or other stable habitat. Habitat availability less than desirable.	**(6-10)**	___
Less than 10% rubble, gravel, or other stable habitat. Lack of habitat is obvious.	**(0-5)**	___

Pool substrate characterization[c]

Mixture of substrate materials with gravel and firm sand prevalent; root mats and submerged vegetation common.	**(16-20)**	___
Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	**(11-15)**	___
All mud or clay or channelized with sand bottom; little or no root mat, or submerged vegetation.	**(6-10)**	___
Hard-pan clay or bedrock; no root mat or vegetation.	**(0-5)**	___

Pool variability[b, c]

Even mix of deep/shallow/large/small pools present.	**(16-20)**	___
Majority of pools large and deep; very few shallow.	**(11-15)**	___
Shallow pools much more prevalent than deep pools.	**(6-10)**	___
Majority of pools small and shallow or pools absent.	**(0-5)**	___

Canopy cover (shading)[c, d, g]

A mixture of conditions where some areas of water surface fully exposed to sunlight, and others receiving various degrees of filtered light.	**(16-20)**	___
Covered by sparse canopy; entire water surface receiving filtered light.	**(11-15)**	___
Completely covered by dense canopy; water surface completely shaded OR nearly full sunlight reaching water surface. Shading limited to <3 hours per day.	**(6-10)**	___
Lack of canopy; full sunlight reaching water surface.	**(0-5)**	___

Channel alteration[a]

Little or no enlargement of islands or point bars, and/or no channelization.	**(12-15)**	___
Some new increase in bar formation, mostly from coarse gravel; and/or some channelization present.	**(8-11)**	___

Moderate deposition of new gravel, coarse sand on old and new bars; and/or embankments on both banks.	**(4-7)**	___
Heavy deposits of fine material, increased bar development; and/or extensive channelization.	**(0-3)**	___

Deposition[c]

Less than 5% of bottom affected; minor accumulation of coarse sand and pebbles at snags and submerged vegetation.	**(12-15)**	___
5-30% affected; moderate accumulation of sand at snags and submerged vegetation.	**(8-11)**	___
30-80% affected; major deposition of sand at snags and submerged vegetation; pools shallow, heavily silted.	**(4-7)**	___
Channelized; mud, silt, and/or sand braided or nonbraided channels; pools almost absent due to deposition.	**(0-3)**	___

Channel sinuosity[b]

Instream thalweg channel length 3 to 4 times straight line distance.	**(12-15)**	___
Instream thalweg channel length 2 to 3 times straight line distance.	**(8-11)**	___
Instream thalweg channel length 1 to 2 times straight line distance.	**(4-7)**	___
Channel straight; channelized waterway.	**(0-3)**	___

Lower bank channel capacity[b]

Overbank (lower) flows rare. Lower bank W/D ratio <7.	**(12-15)**	___
Overbank (lower) flows occasional. W/D ratio 8-15.	**(8-11)**	___
Overbank (lower) flows common. W/D ratio 15-25.	**(4-7)**	___
Peak flows not contained or contained through channelization. W/D ratio >25.	**(0-3)**	___

Upper bank stability [a]

Upper bank stable. No evidence of erosion or bank failure. Side slopes generally <30 percent. Little potential for future problems.	**(9-10)**	___
Moderately stable. Infrequent, small areas of erosion mostly healed over. Side slopes up to 40 degrees on one bank. Slight potential in extreme floods.	**(6-8)**	___
Moderately unstable. Moderate frequency and size of erosional areas. Side slopes up to 60 degrees on some banks. High erosion potential during extreme high flow.	**(3-5)**	___
Unstable. Many eroded areas. "raw" areas frequent along straight sections and bends. Side slopes >60 degrees common.	**(0-2)**	___

Bank vegetation OR Grazing or other disruption[d]

Over 90% of the streambank surfaces covered by vegetation.	**(9-10)**	___
70-89% of the streambank surfaces covered by vegetation.	**(6-8)**	___
50-79% of the streambank surfaces covered by vegetation.	**(3-5)**	___
Less than 50% of the streambank surfaces covered by vegetation.	**(0-2)**	___

Appendix 3. (Continued).

OR

Vegetative disruption minimal or not evident. Almost all potential plant biomass at present stage of development remains.	**(9-10)**	___
Disruption evident but not affecting community vigor. Vegetative use is moderate, and at least one-half of the potential biomass remains.	**(6-8)**	___
Disruption obvious; some patches of bare soil or closely cropped vegetation is present. Less than one-half of the potential plant biomass remains.	**(3-5)**	___
Disruption of streambank vegetation is very high. Vegetation has been removed to 2 inches or less in average height.	**(0-2)**	___

Streamside cover[b]

Dominant vegetation is mixture of tree and shrub.	**(9-10)**	___
Dominant vegetation is of tree form.	**(6-8)**	___
Dominant vegetation is grass or forbes.	**(3-5)**	___
Over 50% of the streambank has no vegetation and dominant material is soil, rock, bridge material, culverts, or mine tailings.	**(0-2)**	___

Riparian vegetative zone width (least buffered side)[e, f, g]

>18 meters	**(9-10)**	___
Between 12 and 18 meters.	**(6-8)**	___
Between 6 and 12 meters.	**(3-5)**	___
<6 meters.	**(0-2)**	___

Total ___

[a]From Ball 1982.
[b]From Platts et al. 1983.
[c]From EPA 1983.
[d]From Hamilton and Bergersen 1984.
[e]From Lafferty 1987.
[f]From Schueler 1987.
[g]From Bartholow 1989.

Appendix 4. Habitat Assessment Protocol Physical Characterization/Water Quality Data Sheet.

Date:	Analyst:	Station #:	Location:

Physical Characterization

Riparian Zone/Instream features

Predominant Surrounding Land Use:
Forest Field/Pasture Agriculture Residential Commercial
Industrial
Other____
Local Watershed Erosion: None Moderate Heavy
Local NPS Pollution: No evidence Some potential Obvious
Estimated Stream Width____________________ m
Estimated Stream Depth Riffle______m Run______m Pool______m
High Water Mark___________m Velocity__________m/s
Dam present Yes___ No___ Channelized Yes___ No___
Canopy Cover: Open Partly open Partly shaded Shaded

Sediment/Substrate

Sediment Odors: Normal Sewage Petroleum Chemical Anaerobic
None Other______________________________
Sediment Oils: Absent Slight Moderate Profuse
Sediment Deposits: Sludge Sawdust Paper Fiber Sand
Relict Shells Other______________________________
Are the underside of stones which are deeply embedded black?
Yes______ No______

Inorganic Substrate Components

Substrate Type	Diameter	% Composition in sampling area
Bedrock		
Boulder	>256 mm (10 inches)	
Cobble	64-156 mm (2.5-10 inches)	
Gravel	2-64 mm (0.1-2.5 inches)	
Sand	0.06-2.00 mm (gritty)	
Silt	0.004-0.06 mm	
Clay	<0.004 mm (slick)	

Appendix 4. (Continued).

Organic Substrate Components

Substrate Type	Characteristic	% composition in sampling area
Detritus	Sticks, Wood, Course Plant Material (CPOM)	
Muck-Mud	Black, Very Fine Organic (FPOM)	
Marl	Grey, Shell Fragments	

Water Quality

Temperature_______C Dissolved Oxygen______ppm pH______
Conductivity_______ Other_______________________________
Instruments used:_______________________________________

Stream Type: Coldwater Warmwater
Water Odors: Normal Sewage Petroleum Chemical None Other__________
Water Surface Oils: Slick Sheen Globs Flecks None
Turbidity: Clear Slightly turbid Turbid Opaque
Water Color:__________________________

Photograph Number:________

Weather Conditions

Observations And/Or Sketch

Chapter 5

SAMPLING PROTOCOL FOR THE RAPID BIOASSESSMENT OF LOTIC MACROINVERTEBRATE COMMUNITIES IN MISSOURI

INTRODUCTION

This chapter explains procedures and provides guidelines for collection, preservation, identification, recording, and analysis of macroinvertebrate samples.

FIELD METHODS

Collection and Preservation

Methods presented here are intended for use only in streams that are considered wadeable, usually less than an average of 1.5 m deep. Sampling protocol can be adapted for use in the accessible, shallow portions of larger streams. Sampling should be done only when flow conditions do not impair the ability of the investigator to efficiently collect organisms from all major habitats. Ideally, sampling efforts should be carried out during periods of stable base flow and temperature. For example, in Arkansas the optimum sampling periods that correspond to stable flow and temperature are generally from February through March and from July through September (Shackleford 1988). The most appropriate sampling periods for Missouri are believed to be during similar times.

This protocol is a synthesis of methods described in the EPA Rapid Bioassessment Protocols for Use in Streams and Rivers (Plafkin et al. 1989) and the North Carolina Division of Environmental Management, Water Quality Section, Protocols (Lenat 1988). Emphasis is placed upon a multihabitat sampling approach where particular habitat types are sampled, stored, and processed individually. Thus, samples collected from each site are not composites, which provides the ability to factor out habitat differences between sites. A habitat was not sampled in a particular stream or river unless it was commonly found. This will enhance comparisons involving streams where all habitats are not present.

Once suitable sites were identified, macroinvertebrate collection could begin. Materials required for sampling included: a bottom aquatic kicknet with an 18 x 8" frame and 800 x 900 mμ mesh net (Wildlife Supply Company, Saginaw Michigan); a 20 x 14 x 5" clear plastic tub (sample concentrating unit [SCU]); an 18 x 8" bag sewn from 500 mμ Nitex; a nylon scrub brush; a 4" brine shrimp net; a littoral sample wash bucket (Wildco); a plastic bucket; 1 qt. wide-mouth mason jars (an average of five per sampling station); and 10% formalin solution.

The SCU was made from a plastic pan large enough to accommodate the bottom aquatic kicknet. A 0.25" mesh wire screen was placed over the pan to retain large debris and allow the sample to pass through. All large debris from the wire screen and the SCU should be discarded after being washed off and searched for attached organisms. The net should also be checked for clinging organisms which should be added to the composite sample if found.

Field preservation of the sample was accomplished by pouring excess water from the SCU through a 500 mμ mesh sieve or brine shrimp net. All organisms and detritus that were retained could be backflushed into the sample container with a small amount of formalin preservative. Backflushing was most effective if the sieve or net fit into the sample container. Using a small squeegee, the remaining sample was concentrated into a corner of the SCU. From there the sample could be scooped into a sample container making sure that sufficient space remained for preservative. The sample was covered with a preservative composed of rose bengal stain at a concentration of 50 mg/L in 10% formalin (Mason and Yevich 1967). The formalin solution needed to be replaced in 48 hours if the sample contained large amounts of organic matter.

A simple device that facilitated changing the formalin solution can be made from a 2" section of PVC pipe slightly larger in diameter than the sample container. Nitex cloth (500 mμ mesh) was attached to the bottom of the ring. When changing formalin, the ring was placed over the sample jar, inverted, and drained. Replacement formalin was poured directly through the nitex and backflushed the organisms into the container.

Procedures common to the sampling of all habitats included a "dry run" through the station prior to sampling, to observe characteristic habitat conditions. This was followed by a return trip during which collections of a specified number of replicate habitat samples were taken from areas of designated size proportion. Final samples for a particular habitat consisted of the combined contents of all replicate samples collected from that habitat. Individual habitats sampled during this project and their specific sampling protocols are listed below.

Types of Habitats Sampled

1. Flowing Water (coarse substrates) Cs flow

Cs flow habitats are commonly called riffles and runs (glides). Riffles are shallow, turbulent stream segments with higher gradients than pools or runs (glides). Runs (glides) are moderately shallow stream channels with laminar flow, and lacking pronounced turbulence. Sampling was done with the bottom aquatic kicknet. Approximately 1 m^2 of substrate was disturbed, by the collector's feet or a three pronged hand cultivation tool, to a depth of 15 cm. All large pieces of course substrate were brushed and washed off, allowing the current to carry organisms into the net. A total of six disturbances from a variety of depths, current velocities, and coarse substrate mixtures were collected and composited into the SCU.

2. Flowing Water (fine substrate)

Sand and silt substrates in areas of measurable current velocity were sampled using the bottom aquatic kicknet. Approximately 1 m^2 of substrate was sampled by placing the kicknet downstream of the sample location and vigorously disturbing it to a depth of 15 cm by using a foot shuffling action. Twelve samples, from areas with a variety of depths, velocities, and organic contents, were collected and composited into the SCU half full of water. A "stir and pour" elutriation technique was used to separate the organisms from the residual fine substrate until no organisms were observed in substrate. The elutriate was poured through a 500 mμ sieve or a brine shrimp net. Organisms retained in the mesh should be field preserved by backflushing the contents with a 10% formalin solution into a sample container. This habitat was rarely encountered in the Ozarks, but was very common in the Lowland and Prairie streams.

3. Non-flowing Water

This habitat was defined as depositional areas including forewaters, backwaters, and edgewaters with no appreciable flow. Nonflowing substrate sampling was done with a bottom aquatic kicknet. Collections were made in a variety of the microhabitats. An approximate 1 m^2 area of substrate was disturbed using the foot shuffling method. To do this the substrate was disturbed by the collector's feet to a depth of 25 cm and organisms that were suspended in the water column were collected by sweeping the net back and forth at a short distance over the substrate. Three passes were made for each net sample at the end of which the kicknet was again swept through the area to capture any dislodged organisms which had failed to be captured in the original passes. During various phases of kicknet sweeping it was sometimes necessary to delay sweeps by removing the kicknet from the water and shaking excess water through the net in order to assure that water currents generated by sweeping were passing through the net rather than backing up around the net because of clogging. A total of six net samples were made and composited into the SCU. The net was then checked for clinging organisms, to be added to the composite, and all large pieces of debris were washed and checked for organisms. The sample was then poured into a sieve bucket (533 mμ mesh) and washed if a large amount of sediment was present. The remaining sample could then be backflushed into the SCU, concentrated, and preserved as described previously.

4. Macrophyte

Sampling aquatic vegetation was done with the bottom aquatic kicknet. Both emergent and submergent vegetation was sampled if present. The investigator sampled six areas of approximately 1 m^2 each.

In areas with current, the net was placed downstream of the target area and individual plant portions were shaken, in an upstream-to-downstream manner, to dislodge organisms into the current and, subsequently, into the net. In vegetated areas with no appreciable flow, sampling was best accomplished by two people, one to hold the net and shake vegetation and another to produce a false current into the net. In all cases, care was taken not to disturb the underlying substrate in order to prevent inclusion of atypical organisms within the sample. Terrestrial invertebrates from emergent portions of the vegetation were, however, frequently captured. Removal of floating arachnids was possible during field processing, but other terrestrials had to be retained and became the concern of the taxonomist. Procedures performed after collection of each replicate sample were identical to those described for previous habitats.

Samples were composited into the SCU and all large pieces of plant material and debris vigorously washed, checked for clinging organisms, and removed. The sample was then concentrated and preserved as described previously.

5. Leaf Packs and Small Woody Debris

Leaf packs and accumulations of woody debris that collected on snags or rocks in areas of flowing water were collected. Leaf packs could be a major habitat in streams during the late winter sampling period but not available during the summer sampling period. Samples were collected by grabbing six handfuls of the material and placing them into a large plastic pan for processing. Large pieces of debris and leaves were washed, removed, and then discarded after being searched for attached organisms. The sample was then concentrated and preserved as described previously. This habitat was sampled during initial collections of 1993, but excluded from subsequent sampling due to the low numbers and diversity of organisms found and the high effort required to process samples.

6. Snag

Organisms associated with logs and growths of periphyton or moss on logs were collected by vigorously brushing 12 areas of approximately 600 cm^2 each (6 x 18") using a nylon scrub brush. When the target area was in current, one person would hold the bag open, downstream of the snag, and the other would scrub the surface with the brush, repeatedly, in a direction most likely to force detached organisms into the Nitex bag. If the snag material was originally located out of the current, and the piece was small enough for the scrubber to carry, then this material was moved to the current and a portion was placed within, or upstream of the bag for scrubbing. If it was desirable to sample larger material from areas without current, then a false current was generated by the scrubber with one hand while the holder positioned the bag down current.

During sampling of this habitat, replicate samples were allowed to accumulate in the bag, with contents not being transferred to the SCU until all replicate samples had been collected. Thus, it was found desirable to periodically concentrate accumulated contents into one corner of the bag and to be certain that enough current flowed through the bag during sampling that previously captured organisms had no opportunity to escape. Protocol for this habitat required that 12 replicate areas were sampled from a variety of flow regimes and from woody debris in various stages of decay. The requirement for a larger number of replicates than for other habitats was based upon the relatively low density of invertebrates encountered on snags and the need for adequate sample size. The sample was composited into a SCU by inverting the bag and flushing it with water from a hand held sprayer. Excess water in the SCU was poured off through a 500 mμ sieve or brine shrimp net. The sample was then processed and preserved as described previously.

7. Undercut Banks and Rootmats

For our purposes only fine, densely clumped, feeder roots were sampled, as coarser root portions have significantly less surface area and were considered as inferior habitat. A rootmat sample consisted of six replicate samples, each taken from an area of 1 m of stream bank length as measured by two widths of the kicknet frame. Due to the patchiness of this habitat, it was frequently necessary to modify the protocol and collect a larger number of replicate samples from smaller areas, until a total of 6 m of stream bank had been sampled.

Sampling of rootmats was accomplished using a number of collection techniques. If the replicate sample was to be taken from an area within the current, a kicknet was placed downstream of the sampling area and the target material was shaken by hand or foot, in an upstream–to–downstream manner, so that organisms were dislodged and driven by water currents into the net. If the target habitat happened to be in an area of little or no flow, and the stream bank was steep enough that the net could be maneuvered under the habitat, then a "lift and shake" approach was employed. This involved lifting the habitat material, as supported by the kicknet frame, and vigorously shaking the material up and down at the water surface to dislodge and capture invertebrates. Once four or five quick shakes had been made, the net was immediately removed from beneath the habitat and swept rapidly back and forth through the water column to capture any remaining, dislodged organisms. In areas without current, where conditions were such that the kicknet could not effectively be used to "lift and shake" other techniques were appropriate. Under these conditions, the kicknet was place alongside habitat materials, the materials were vigorously shaken within the water, and false currents were immediately produced by hand to force dislodged organisms into the net. Another option was lifting materials by hand, into the net, and shaking them at the water surface. The sample was then concentrated and preserved as previously described.

Field Processing

Collections from all habitat types were field processed in identical fashion. First the SCU and its contents were taken to an appropriate base location within the sampling station (usually a point bar) where field processing hardware was located. To begin the process of removing invertebrates from the SCU for preservation, stream water was collected in a plastic bucket and poured into the SCU to a level which would allow the washing of any large debris contained within the sample. Care was taken not to overfill the device to a point where water could not later be drained off easily. Once flooded with water, the contents of the SCU were inspected by hand for individual pieces of large consolidated debris such as twigs, bark, large stones, and undecomposed leaf matter. Once located, each piece was grasped and vigorously agitated within the SCU to dislodge clinging organisms. Once the debris was rinsed, visually inspected, and found to be free of invertebrates, it was removed from the sample in order to minimize the volume of materials to be preserved.

When all large materials had been removed, the SCU was lifted by hand and swirled vigorously in a circular motion so that the organic fraction of the sample became suspended above any heavier inorganic debris, such as small stones or sand, which remained in the bottom of the SCU. After swirling, the water fraction within the SCU was quickly poured from a corner of the device into a brine shrimp net which had previously been laid across the top of a wash bucket so that any water passing through the net would enter the bottom of the bucket. By this technique, organic debris and invertebrates were removed from the SCU. Once the brine shrimp net was nearly filled with debris, pouring was ceased and the net was shaken vigorously, in a rocking motion, so that the majority of water adhering to the organic matter was removed, while the debris itself was retained. The net was then inverted over the mouth of a collection jar for transference of the sample. Once transference of the net's contents was completed, the net was returned to its position astraddle the wash bucket. Remaining contents of the SCU were then agitated again and poured into the net, adding more water as necessary, until all organic matter had been transferred into collection jars. In cases where large quantities of organic debris were present in the sample, as often happened in backwater habitats, it was desirable to speed up the transfer process by slowly pouring off the water in the SCU so that the majority of organic debris remained in the device where it could be scooped by hand or by spatula directly into collection jars. This technique required rinsing of hands and spatula into the SCU, after transference, to avoid losing organisms. Then the SCU was flooded, and remaining debris was removed by the previously described brine shrimp net method. Transference of organisms from the SCU was considered complete when no organic matter remained visible in the unit when agitated while containing water. When this qualification was met, the device was allowed to remain stationary, while flooded, and was observed for 2 minutes for signs of movement from biota. If after this period no organisms were visible, the remaining inorganic fraction within the SCU was disposed of and the SCU was ready for sampling another habitat. Inevitably, during processing, some organic matter and invertebrates failed to enter the brine shrimp net and ended up in the bottom of the wash bucket. In order to transfer this portion of the sample to collection jars, it was necessary to move the wash bucket into the stream channel and concentrate the contents into one corner by repeatedly dipping the bucket into the water at progressively larger angles to the vertical. Contents could then be poured into a brine shrimp net placed against the outer lip of the bucket's rim.

Once all materials were loaded into collection jars, the jars were labeled inside and out with sampling date, sampling location, and respective habitat unit. In cases where filamentous algae were present in a sample, or where hand transference of material was performed, collection jars frequently contained free water, at this point, which had to be removed in order to avoid dilution of the preservative solution. This was accomplished, prior to preservation, by placing the jar lid over the mouth of the jar, so as to leave a small opening for water passage, and inverting the jar. Collection jars were then filled with formalin solution to a level slightly above that of sample contents and stored for later laboratory processing procedures. At this point, field collection and processing of a habitat was complete.

Length of Stream Reach to be Sampled

All macroinvertebrate sampling was done in a stream reach approximately 20x the average stream width. This length of stream will encompass approximately two riffle sequences (10-14 stream widths) or meander sequences (14-20 stream widths) according to Hynes (1970). Currently there is no quantitative evidence to support the selection of this distance to ensure collection of a majority of taxa. The multihabitat sampling protocol employed by the North Carolina Division of Environmental Monitoring specifies no limitations on the length of stream reach to sample within. However, Mr. David Lenat, North Carolina Division of Environmental Monitoring (personal communication) found that two riffle/pool sequences were normally sampled by three people within a 2-hour period.

Collection of Water Samples

Aside from invertebrate collections and habitat analyses, it was also necessary during all sampling periods, except spring of 1994, to collect and analyze water samples from each sampling station for purposes of site verification and data analysis. In all cases, these collections were made immediately after a season's invertebrate sampling was completed. This period was chosen so that water samples would best reflect the conditions present during invertebrate sampling while still allowing samples to be returned to the laboratory as quickly as possible for analysis.

Water sampling protocol called for collection of subsamples across a transect drawn through a stream run. Subsamples were collected in a 250 mL container and then transferred to a 1-L cubetainer until the cubetainer was full. The container was then sealed, externally labeled with collection date and station code, and placed on ice. One portion of each water sample was filtered and placed on ice to later be analyzed for dissolved chemical constituents. The unfiltered portion of each sample was retained for analysis of total chemical constituents. At the end of each collection run, water samples were taken to the Fisheries and Wildlife Limnology Laboratory at the University of Missouri–Columbia, and frozen until chemical analysis could be performed.

LABORATORY PROCESSING OF INVERTEBRATES

Subsampling

A pilot study in North Carolina compared 100 organism vs. 300 organism subsamples (Plafkin et al. 1989). It was determined that 100 organisms were adequate for making a good evaluation of water quality, even at the family level of identification. A 100 organism subsample has also proven adequate in numerous other studies for impact detection (Hilsenhoff 1982, 1987, Nuzzo 1986, Bode 1988). A subsampling method that is modified (Caton 1991) from that of Plafkin et al. (1989) was used to allow rapid isolation of the 100 organisms.

Invertebrate Processing

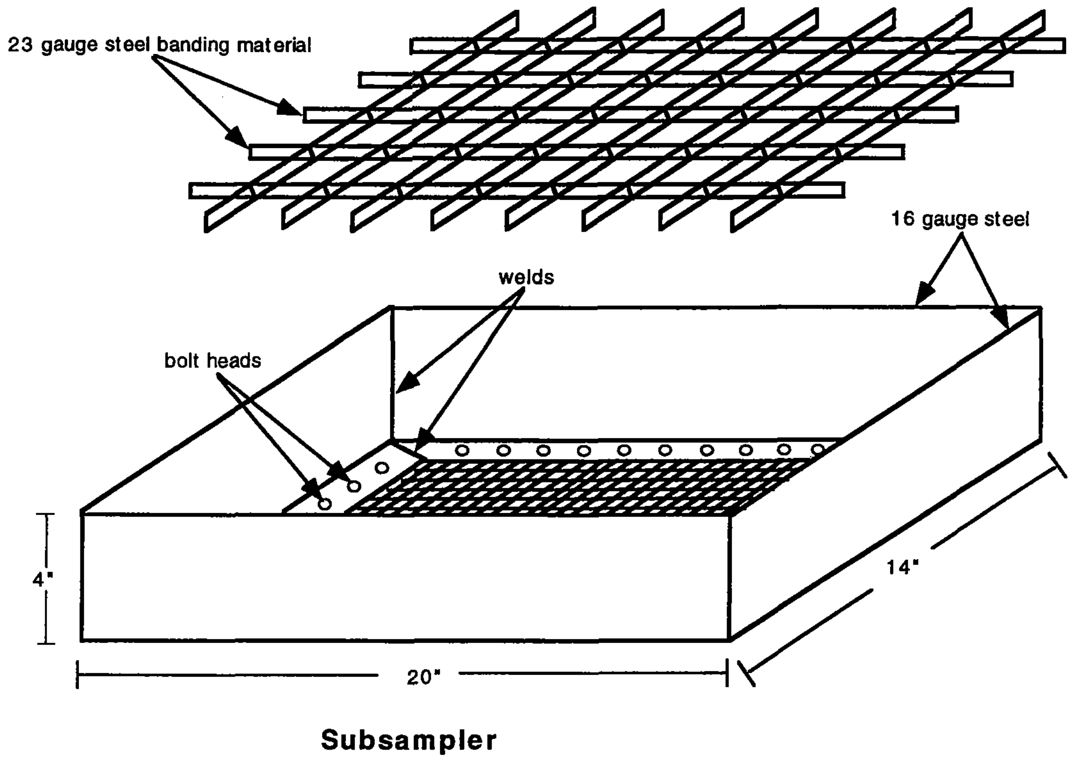
Materials required for this process included: a "mason jar sieve" composed of PVC pipe and 500 mμ Nitex (Fig. 1); a subsampling device created from a modified design of a Wildco wash frame, with a removable grid with 70 2X2" painted squares (Figs. 1 and 2); a 20 x 14 x 5" clear plastic tub; a 300 mμ mesh U.S. Geological Survey (USGS) sieve; a 10,000 mμ mesh USGS seive; a small paint brush; and a spatula, approximately 1 1/2" wide.

With these materials at hand, laboratory processing began with the draining of formalin solution from a collection jar. This was accomplished by opening the jar, placing the mason jar sieve over the jar's mouth, and inverting to allow drainage of waste formalin into an appropriate disposal container. The subsampling device was then placed into a sink, and the contents of the collection jar and the mason jar sieve were rinsed with water into the device. The process was continued, until all jars containing the contents of a specific habitat from a collection station had been drained and emptied. At this time, the contents of the subsampling device were rinsed with water until all detectable formalin solution was removed. The subsampling device was then placed within the plastic tub, and the tub was placed into the sink and filled with water to a level which would allow stirring of the contents of the subsampling device. These materials were then agitated by hand for a period of time adequate to randomly disperse organisms and to uniformly distribute detritus throughout the device. If, during this process, any large pieces of debris were present, they were rinsed within the device, checked for clinging organisms, and removed. Once the above steps were completed, the subsampling device was removed from the plastic tub to drain, and the tub was emptied of water.

Once drained, the subsampling device was placed back into the tub, the removable grid was placed within the device, and three grid squares were randomly selected. The contents of these squares were then removed from the subsampling device by sweeping them with a small paint brush onto a narrow spatula. Depending upon the types and quantities of materials within the subsampling unit, this process could be accomplished with the grid in place or, when necessary, indentations could be made within the underlying detritus, around the perimeter of the target grid sections, and the grid then removed. In cases where coarse root or leaf materials was present in the unit, it was sometimes necessary to cut these materials along grid lines, with scissors, before removal of a section's contents. If, during the removal of contents from the device, large organisms, such as crayfish, were positioned across grid lines, the organism was considered the property of the section which contained the largest portion of the organism. Once the contents of the designated grid sections were removed, they were placed into a single container of a size suitable to accommodate the subsample.

At this point, the subsampling device was set aside and the subsample was rinsed from the storage container into the two USGS sieves, which had previously been stacked and placed in the sink. Once all subsampled materials were inside the sieves, the materials were rinsed with water, so that smaller organisms and debris were washed into the bottom, 300 mμ mesh sieve, while coarser debris and larger organisms remained in the upper sieve. The sieves were then unstacked, the upper sieve was set aside, and the contents of the lower sieve were rinsed into one corner for removal. Using a spoon to scoop materials, and water to rinse the remainder, materials from the bottom sieve were then transferred into the original storage container. During this process as little water was used for rinsing, as possible, in order to speed later removal of invertebrates from the subsample. When the bottom sieve was emptied, it was inverted and placed over the larger mesh sieve, and both sieves were inverted again and replaced in the sink. The contents of the upper sieve were then rinsed into the finer sieve, and these materials were transferred to a separate watertight container by the method previously described.

Once subsample contents were separated into finer and coarser portions, the container with the fine fraction was taken to a work table containing a binocular dissecting microscope and a modified zooplankton wheel. A portion of the fine sample was then transferred, by spoon, to the reception channel of the zooplankton wheel and the wheel was placed upon the microscope stage, where the sample was observed under 30X magnification. When invertebrates were located, they were removed from the sample



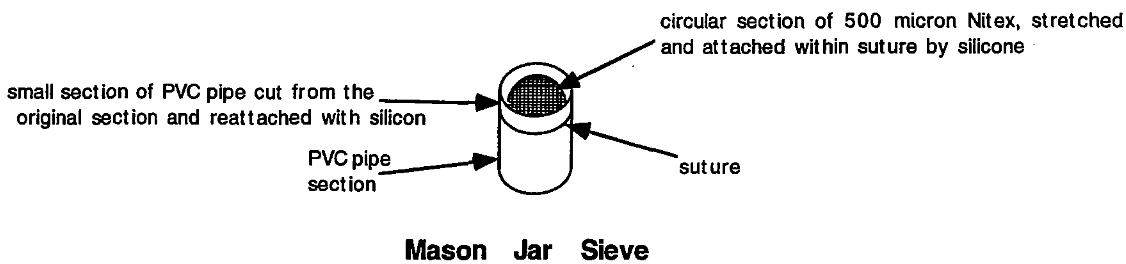


Fig. 1. Illustration of the subsampler and the mason jar sieve used for laboratory processing of invertebrates.

Subsampler (bottom view)

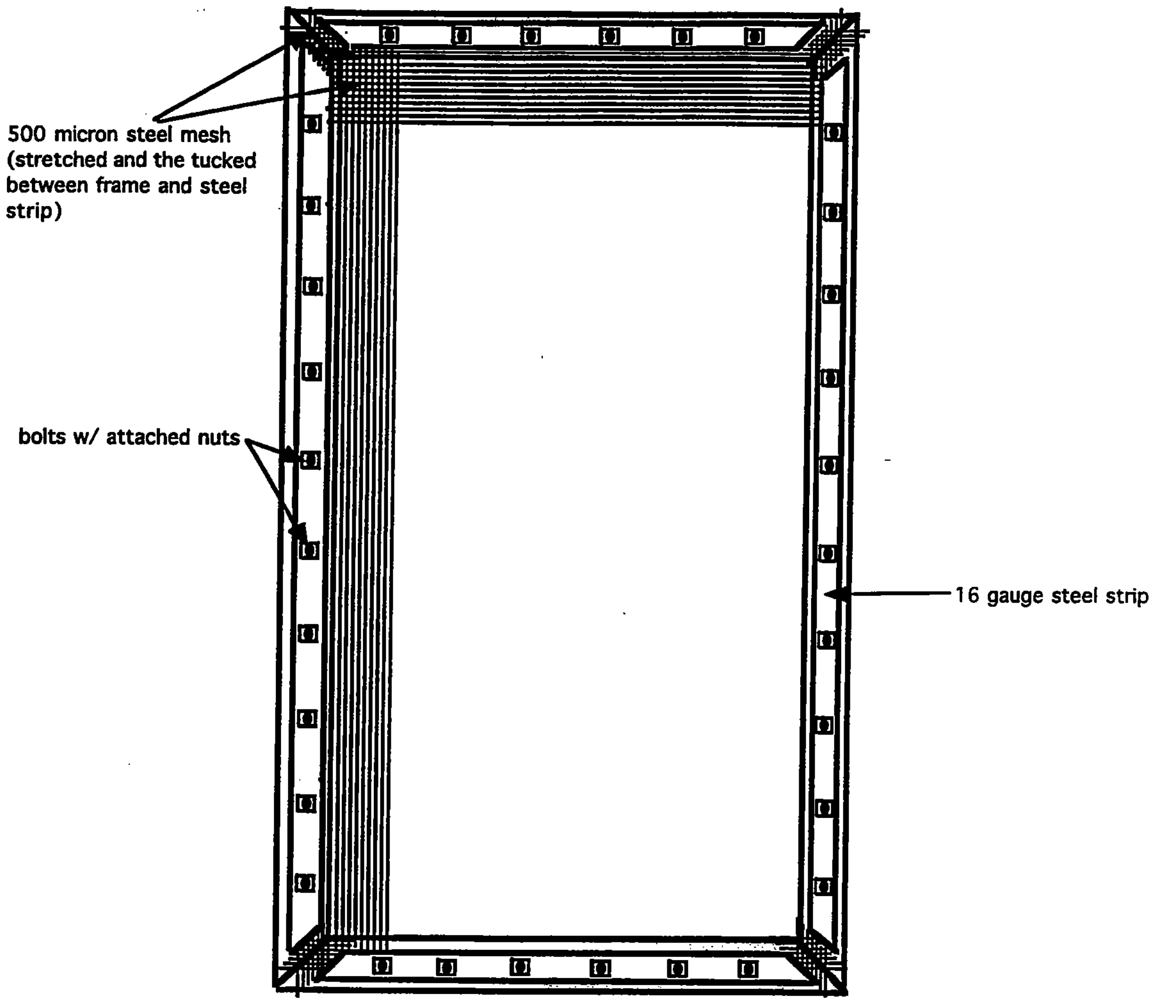


Fig. 2. Bottom view of subsampler illustrating some construction details.

and placed in vials containing a solution of 80% ethanol. As invertebrates were removed from the subsample, a running total of their number was also maintained.

Once the entire fine fraction of the original subsample was inspected in this manner, the zooplankton wheel was removed from the microscope. The coarser fraction of the subsample (which could not be effectively loaded into the zooplankton wheel) was then transferred to a small, shallow plastic pan, and the pan was placed upon the microscope stage. Individual pieces of large debris were then inspected for clinging organisms and removed from the pan. Invertebrates removed from this portion of the subsample were retained and tallied as previously described.

If, after processing, the original habitat subsample of three grid sections was found to have contained more than 100 invertebrates, then subsampling for this habitat unit was considered complete. If fewer than 100 organisms had been removed, however, the contents of additional grid sections were randomly removed from the subsampling device, one at a time, for processing, until 100 or more invertebrates were obtained. Upon completion of the subsampling process, collection vials were labeled internally with the sampling date, sampling location, and habitat unit. The vials were then sealed and the remaining contents of the subsampling device discarded. A record was also made of the number of grid sections which were processed for each habitat at each sampling station. This information would later be used for weighting of data, as deemed necessary for calculations of some community metrics. The average time required for removal of invertebrates from a habitat sample was 2 hours.

Identification and Recording

Identification

Identifications were made to the lowest possible taxonomic level. A Taxonomic Bibliography (Appendix 1), in which an attempt has been made to include the most current revisions and updates, has been included. All organisms that are kept as part of the reference collection will receive expert confirmation. Reference collections will then be maintained in the University of Missouri-Columbia Wilbur R. Enns Entomology Museum, and The School of Natural Resources Fisheries Museum.

Most insects collected during this study were identified to genus level using keys provided in Merritt and Cummins (1984). Plecoptera were further identified to species level (Poulton and Stewart 1991) as were Ephemeroptera of the genus *Stenonema* (Bednarik and McCafferty 1979, McCafferty 1981). Some additional mayfly genera, collected during spring and fall of 1993, were also identified to species; but these same genera, collected during subsequent sampling periods, were identified to generic level only. These included the genera *Ephemerella* (Allen and Edmunds 1965); *Eurylophella* (Allen and Edmunds 1963b); *Serratella* (Allen and Edmunds 1963a); *Isonychia* (Kondratieff and Voshell 1984); *Baetisca* (Pescador and Berner 1981); and *Caenis* (Provonsha 1992).

Larval Chironomidae collected during spring of 1994 were keyed to family level only, but were identified to genus level (Merritt and Cummins 1984) during all other sampling periods. For identification purposes, slide mounting of larva was performed as described in Merritt and Cummins (1984); with the exception that whole-larva mounting was performed on all but the largest specimens. This technique was found to be adequate for generic level identifications and was faster than techniques requiring separation of the heads and bodies of larvae.

With regard to the noninsect taxa, Crustacea were identified to genus level using Pennak (1989). Gastropoda and members of the subclass Hydracarina collected during 1993 were identified to genus (Pennak 1989), but were not keyed beyond phylum and subclass, respectively, in subsequent collections. During all sampling periods, members of the phylum Annelida were identified to class (Pennak 1989). Molluscs collected during sampling were released unharmed during field processing, due to inherent sampling biases and the imperiled status of many member species.

Once identified, all individuals of a given taxon are readied for permanent storage. Permanent storage for most organisms consists of placing them in a sample vial filled with 70% alcohol and inserting an internal label with the name of the waterway, county, map coordinates, date, habitat, and name of the analyst. A separate label containing taxonomic identification is also inserted. Chironomidae and Oligochaeta were permanently mounted for identification on microscope slides with CMCP-10 mounting media (Poly Sciences Inc., Paul Valley Industrial Park, Warrington, PA). Each slide was labeled with the same information as the alcohol vials and placed into a slide box maintained exclusively for that station. A Macroinvertebrate Laboratory Bench Sheet (Appendix 2) containing identification, date, location, and enumeration of all samples was completed for each station.

Recording Data

Data for each sample habitat type was recorded in the appropriate column on the laboratory bench sheet. Each taxon was listed only once in the left hand column, so that the total number of taxa could easily be determined and entered into the appropriate space. The laboratory bench sheet was constructed in a flexible manner to enable the analyst to use the composite data (left hand column) or to use individual habitats. It could be helpful to highlight any unique taxa to a particular habitat in order to facilitate an understanding of habitat requirements.

Appendix 1. Taxonomic bibliography, macroinvertebrate sampling protocol.

1. **General References**

McCafferty, W. P. 1981. Aquatic Entomology. Science Books International, Boston, MA. 448pp.

Merritt, R. W. and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. Third Edition. Kendall/Hunt Publishing Company, Dubuque, IA.

Pennak, R. W. 1978. Freshwater invertebrates of the United States. Second Edition. John Wiley and Sons, Inc., New York, NY. 803pp.

Pennak, R. W. 1989. Freshwater invertebrates of the United States—Protozoa to Mollusca. Third Edition. John Wiley and Sons, Inc., New York, NY. 628pp.

2. **Annelida - Oligochaeta**

Brinkhurst, R. O. 1975. Oligochaeta. Pages *69-85 In* F. K. Parrish (ed.). Keys to water quality indicative organisms of the southeastern United States. Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH.

Brinkhurst, R. O. 1981. A contribution to the taxonomy of the Tubificidae (Oligochaeta:Tubificidae). Proceedings of the Biological Society of Washington 94(4):1042–1067.

Brinkhurst, R. O. 1982. Oligochaeta. Pages 50–61 *in* S. P. Parker (ed.), Synopsis and classification of living organisms, Vol. 2. McGraw-Hill Book Co., New York, NY.

Brinkhurst, R. O. and R. D. Kathman. 1983. A contribution to the taxonomy of the Naididae (Oligochaeta) of North America. Canadian Journal of Zoology 61:2307-2312.

3. **Annelida - Hirudinea**

Klemm, D. J. 1982. Leeches (Annelida:Hirudinea) of North America. EPA-600/3-82-025. Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH. 177pp.

Klemm, D. J. 1985. Freshwater leeches (Annelida:Hirudinea). Pages 70-173 *in* D. J. Klemm (ed.), A guide to the freshwater annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co., Dubuque, IA.

4. **Coleoptera**

Brown, H. P. 1972. Aquatic dryopoid beetles (Coleoptera) of the United States. Water Pollution Control Research Series 18050 ELD04/72 Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH.

White, D. S., W. U. Brigham and J. T. Doyen. 1984. Aquatic coleoptera. Pages 361–437 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America (Second Edition). Kendall/Hunt Publishing Co., Dubuque IA.

5. **Crustacea**

Barnard, J. L. and G. S. Karman. 1980. Classification of gammarid Amphipoda. Crustaceana Suppl. 6:5-16.

Hobbs, H. H. Jr. 1976. Crayfishes (Astacidae) of North and Middle America. Water Pollution Control Research Series 18050 ELD05/72, Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH.

Holsinger, J. R. 1976. The freshwater amphipod crustaceans (Gammaridae) of North America. Water Pollution Control Research Series 18050 ELD04/73, Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH.

Williams, A. B. 1954. Speciation and distribution of the crayfishes of the Ozark Plateau and Quachita Provinces. Kansas Univ. Science Bulletin 36(12):803-918.

Williams, W. D. 1976. Freshwater isopods (Asellidae) of North America. Water Pollution Control Research Series 18050 ELD05/72, Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH.

Diptera - Chironomidae

Simpson, K. W. and R. W. Bode. 1980. Common larvae of Chironomidae (Diptera) from New York State streams and rivers with particular reference to the fauna of artificial substrates. New York State Museum Bulletin 439:1-105.

Wiederholm, T. (ed.). 1983. Chironomidae of the holarctic region. Keys and diagnoses. Part I. Larvae. Entomol. Scand. Suppl. 19:1-457.

Wiederholm, T. (ed.). 1986. Chironomidae of the holarctic region. Keys and diagnoses. Part II. Pupae. Entomol. Scand. Suppl. 28:1-482.

7. **Ephemeroptera**

Allen, R. K. and G. F. Edmunds Jr. 1963a. A revision of the genus *Ephemerella* (Ephemeroptera:Ephemerellidae). VI. The subgenus *Serratella* in North America. Annals of the Entomological Society of America 56:583-600.

Allen, R. K. and G. F. Edmunds Jr. 1963b. A revision of the genus *Ephemerella* (Ephemeroptera:Ephemerellidae). VII. The subgenus *Eurylophella.* Canadian Entomology 95:597-623.

Allen, R. K. and G. F. Edmunds Jr. 1965. A revision of the genus *Ephemerella* (Ephemeroptera:Ephemerellidae). VIII. The subgenus *Ephemerella* in North America. Miscellaneous Publication of the Entomological Society of America 4:243–282.

Bednarik, A. F. and W. P. McCafferty. 1979. Biosystematic revision of the genus *Stenonema* (Ephemeroptera:Hepatageniidae). Canadian Bulletin of Fisheries and Aquatic Sciences 201:1-72.

Burks, B. D. 1953. The mayflies, or Ephemeroptera, of Illinois. Bulletin of the Illinois Natural History Survey 26:1–216.

Edmunds, G. F. Jr., S. L. Jensen and L. Berner. 1976. The mayflies of North and Central America. Univ. Minnesota Press, Minneapolis. 330pp.

Kondratieff, B. C. and J. R. Voshell Jr. 1984. The North and Central American species of *Isonychia* (Ephemeroptera:Oligoneuriidae). Transactions of the American Entomological Society 110:129-244.

McCafferty, W. P. 1981. A distinctive new species of *Stenonema.* Proceedings of the Entomological Society of Washington 83(3):512-515.

Needham, J. G., J. R. Traver and Y. C. HS. 1935. The biology of mayflies. Entomological Reprint Specialists, Inc., East Lansing, MI. 759pp.

Pescador, M. L. and L. Berner. 1981. The mayfly family Baetiscidae (Epemeroptera). Part II. Biosystematatics of the genus *Baetisca.* Transactions of the American Entomological Society 107:163-228.

Provonsha, A. V. 1992. A revision of the genus *Caenis* in North America (Ephemeroptera:Caenidae). Transactions of the American Entomological Society 116:801-884.

8. **Hemiptera**

Froeschner, R. C. 1949. Contributions to a synopsis of the Hemiptera of Missouri, Part IV. American Midland Naturalist 42:123-188.

Froeschner, R. C. 1962. Contributions to a synopsis of the Hemiptera of Missouri, Part V. American Midland Naturalist 67:208-240.

Polhemus, J. T. 1984. Aquatic and semiaquatic Hemiptera. Pages 231–260 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America. Second edition. Kendall/Hunt Publ. Co., Dubuque, IA.

9. **Lepidoptera**

Lange, W. H. 1984. Aquatic and semiaquatic Lepidoptera. Pages 348-360 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America. Second edition. Kendall/Hunt Publ. Co., Dubuque, IA.

10. **Megaloptera and Neuroptera**

Evans, E. D. and H. H. Neunzig. 1984. Megaloptera and aquatic Neuroptera. Pages 261-270 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America. Second edition. Kendall/Hunt Publ. Co., Dubuque, IA.

Neunzig, H. H. 1966. Larvae of the genus *Nigronia* Banks. Proceedings of the Entomological Society of Washington 68(1):11-16.

11. **Mollusca**

Buchanan, A. C. 1980. Mussels (Niades) of the Meramec River Basin. Aquatic Series No. 17, Missouri Department of Conservation, Jefferson City. 69pp.

Burch, J. B. 1973. Freshwater Unionacean clams (Mollusca:Pelecypoda) of North America. Biota of freshwater ecosystems identification manual No. 11. U.S. EPA, Washington, DC. 176pp.

Burch, J. B. 1975. Freshwater spharriacean clams (Mollusca:Pelecypoda) of North America. Malacol. Publ., Hamburg, MI. 96pp.

Burch, J. B. 1975. Freshwater Unionacean clams (Mollusca:Pelecypoda) of North America. Malacol. Publ., Hamburg, MI. 204pp.

Burch, J. B. 1982. Freshwater snails (Mollusca:Gastropoda) of North America. PA-600/3-82-026, Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH. 294pp.

12. **Odonata**

Needham, J. G. and M. J . Westfall Jr. 1954. Dragonflies of North America. Univ. California Press, Berkeley and Los Angeles. 615pp.

Westfall, M. J. Jr. 1984. Odonata. Pages 126–176 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America. Kendall/Hunt Publ. Co., Dubuque, IA.

Williamson, E. B. 1932. Dragonflies collected in Missouri. Occasional Papers of the Univ. Michigan Museum of Zoology 240:1-40.

13. **Plecoptera**

Fullington, K. E. and K. W. Stewart. 1980. Nymphs of the stonefly genus *Taeniopteryx* (Plecoptera:Taeniopterygidae) of North America. Journal of the Kansas Entomological Society 53(2):237-259.

Harper, P. P. and K. W. Stewart. 1984. Plecoptera. Pages 182-230 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America. Second edition. Kendall/Hunt Publ. Co., Dubuque, IA.

Hitchcock, S. W. 1974. Guide to the insects of Connecticut. Part VII. The Plecoptera or stoneflies of Connecticut. Bulletin of the State Geological and Natural History Survey 107:1-262.

Poulton, B. C. and K. W. Stewart. 1991. The stoneflies of the Ozark and Ouachita Mountains (Plecoptera). Memoirs of the American Entmological Society #38. 116pp.

Stark, B. P. and A. R. Gaufin. 1976. The nearctic species of *Acroneuria* (Plecoptera:Perlidae). Journal of the Kansas Entomological Society 49(2):221–253.

Stewart, K. W. and B. P. Stark. 1989. Nymphs of North American stonefly genera (Plecoptera). Thomas Say Foundation Series, Volume 12, American Entomological Society, Hyattsville, MD.

14. **Trichoptera**

Morse, J. C. and R. W. Holzenthall. 1984. Tricoptera genera. Pages 312-347 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America. Second edition. Kendall/Hunt Publ. Co., Dubuque, IA.

Schefter, P. W. and G. B. Wiggins. 1986. A systematic study of the nearctic larvae of the *Hydropsyche morosa* group (Trichoptera:Hydropsychidae). Royal Ontario Museum of Life Sciences Miscellaneous Publication M5S 2C6, Ontario, Canada. 94pp.

Shunter, G. A. and D. A. Etnier. 1978. A Manual for the Identification of the Larvae of the Caddisfly Genera *Hydropsyche* Pictet and *Symphitopsyche* Ulmer in eastern and central North America (Trichoptera:Hydropsychidae). EPA–600/4-78-060, Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, OH.

Wigging, G. B. 1977. Larvae of the North American caddisfly genera (Trichoptera). Univ. Toronto Press, Ontario, Canada.

Wigging, G. B. 1984. Tricoptera. Pages 271–311 *in* R. W. Merritt and K. W. Cummins (eds.), An introduction to the aquatic insects of North America. Second edition. Kendall/Hunt Publ. Co., Dubuque, IA.

Appendix 2. Laboratory bench sheet macroinvertebrate sampling protocol.

Date:	Analyst:	Station #:	Location:

Habitat Types

Genus-species	Flow CS	Non-flow	Veg.	Flow FS	Leaf pack	Snag	Under-cut
Total #							
Total # taxa							

Chapter 6

DATA ANALYSIS

SELECTION OF METRICS

A review of the literature supplied a number of measures, indices, or "metrics" useful in describing aquatic communities (Washington 1984, Plafkin et al. 1989, Barbour et al. 1992,). We will use the term metric and define it as a measure of stream health that changes in response to the environmental condition of the stream. Each measure is purported to indicate something about the biotic community, which is related to stream health, at the individual, population, or community level. Several measures used together, if done appropriately, integrate biological response to perturbation, and provide a system to monitor and assess stream health. Eleven such measures were selected for initial investigation of their possible use in Missouri. Measures were selected because of their potential to show a variety of structural and functional responses. Metrics used to evaluate the macroinvertebrate data and their significance are described below.

RICHNESS OF METRICS

Taxa Richness

Total taxa richness reflects the health of the community through a measurement of the variety of taxa (total number of genera or species) present. Total taxa generally increase with improving water quality, habitat diversity, and/or habitat suitability.

Family Richness

The number of different Families of invertebrates reflects the health of the biotic community. Total number generally increases with improving water quality, habitat diversity, and/or habitat suitability.

EPT Index

The EPT index is the sum of all Ephemeroptera, Plecoptera, and Trichoptera taxa and generally increases with increasing water quality. These three orders of aquatic insects are generally considered to be pollution sensitive.

Pinkham and Pearson Similarity Index (PPSI)

Community similarity indices are used in situations where reference communities exist. The reference community can be derived through sampling or prediction for a region through use of a reference database. The PPSI measures the degree of similarity in taxonomic composition in terms of taxon abundance and can be calculated with either percentages or numbers. A weighting factor can be added that assigns more significance to dominant species. See Pinkham and Pearson (1976) and U.S. EPA (1983) for more detail. The formula is:

$$PPSI_{ab} = \text{Sum } \frac{\min(X_{ia},X_{ib})}{\max(X_{ia},X_{ib})} \quad \text{weighting factor} \quad \frac{X_{ia} \times X_{ib}}{\frac{X_a \quad X_b}{2}}$$

where X_{ia},X_{ib} = number of individuals in the ith species in sample a or b.

Percent Model Affinity (PSI)

Percent model affinity compares a test stream to an ideal community, expressed as percent composition of seven major organism groups: Ephemeroptera, Trichoptera, Plecoptera, Diptera, Oligochaetes, Coleoptera, and other (Novak and Bode 1992).

$$PMA = \text{Sum min } (P_{ia}. P_{ib})$$

where P_{ia} is the relative abundance of one of seven faunal groups from the test site, P_{ib} is the relative abundance of the same faunal group in an ideal reference community.

Quantitative Similarity Index for Taxa (QSI)

The QSI compares two communities in terms of presence or absence of taxa, while also taking relative abundance (percent composition) into account. The index is expressed as:

$$QSI = \text{Sum min } (P_{ia}, P_{ib})$$

where:

P_{ia} = the relative abundance of species I at Station A

P_{ib} = the relative abundance of species I at Station B

$\min(P_{ia}, P_{ib})$ = the minimum possible value of species I at Station A or B in terms of relative abundance

Values for this index range from 0 to 100, with identical communities having a value of 100 and totally different communities having a value of 0. In general, values of <65.0 indicate environmental stress, whereas values >65.0 occur as expected variation (Shackleford 1988).

Coefficient of Community Loss (CCL)

This metric measures the loss of taxa from a potentially impacted site when compared to a reference stream

$$CCL = (a-c)/b$$

where a is the numbers of taxa in the reference community, b is the numbers of taxa in a potentially impacted community, and c is the numbers of taxa common to a and b. CCL values exceeding 0.8 are indicative of excessively harmful change in those communities (Courtemanch and Davies 1987). The EPA RBP III (Plafkin et al. 1989) suggested the value of 0.5 as the impairment threshold.

COMMUNITY BALANCE METRICS

Modified Biotic Index (BI)

The index was first developed by Hilsenhoff (1982) and later modified (Hilsenhoff 1987) to summarize overall pollution tolerance of the benthic arthropod community with a single value. It was developed as a means of detecting organic pollution in communities inhabiting rock or gravel riffles.

Each taxa is assigned a tolerance value, related to its assumed tolerance of water quality degradation. The values used in this protocol are based upon Lenat (1993), originally developed for southeastern states. If unavailable from Lenat, values were assigned from Hilsenhoff (1987) and Huggins and Moffett (1988). The formula for the Biotic Index is

$$BI = \text{Sum } (X_I T_I)/n$$

where:

X_i = number of individuals within each species

T_i = tolerance value of that species

n = total number of organisms in the sample

Biotic Index values range from 0 to 10, increasing as the perturbation increases. Although it may be applicable for other types of pollutants, use of the BI in detecting nonorganic pollution effects has not been thoroughly evaluated and is intended for use only with riffle habitat.

Percent Contribution of Dominant Taxon (% Dominant taxon)

Percent contribution of the Dominant taxon to the total number of individuals in a community is a measure of redundancy and evenness and assumes that a highly redundant community (major abundance contributed by a single taxon) reflects an impaired community.

Hydropsychidae/Trichoptera Index

The Hydropsychidae/Trichoptera index is a percentage of Hydropsychidae abundance to total Trichoptera and measures the relative abundance or contribution of this generally mild pollution tolerant Family. For these analyses, Hydropsychidae does not include *Arctopsyche* and *Parapsyche* (Schmid 1968). The Arctopsychids are pollution intolerant; often longer lived than the Hydropsychids; predaceous; and found in higher gradient cold, montane streams (Barbour et al. 1992).

EPT/Chironomidae Index

This ratio summarizes the proportion of the most sensitive taxa—mayflies, stoneflies, and caddisflies—to some of the most pollution tolerant, the Chironomidae; the higher the value the better the water quality.

Shannon's Diversity Index

The Shannon diversity index (Shannon and Weaver 1949) is a measure of community composition which takes into account both richness and evenness. It is assumed that a more diverse community is a more healthy community; diversity increases as the number of taxa increases, and the distribution of individuals among those taxa is evenly distributed. The formula used is

$$H = -\text{Sum } (N_i/N)\ln(N_i/N)$$

where Ni is the number of individuals in the ith taxa sample belonging to the ith species.

Simpson's Diversity Index

This index is a measure of the probability of any two individuals drawn at random from a community belonging to a different taxa. It is based on the proportional abundance of the taxa. It is considered a dominance measure because it is heavily weighted toward the most abundant taxa in the sample while being less sensitive to taxa richness. It is calculated as:

$$D = \text{Sum}\{n_i(n_i - 1)/(N(N-1))\}$$

where:
n_i = the number of individuals in the ith taxa
N = the total number of individuals

FUNCTIONAL FEEDING GROUP METRICS

Ratio of Scrapers/Filterers (S/F)

The ratio of S/F gives a percentage of scraper abundance to the combined total of scrapers and filtering collectors. This metric is considered to be an indication of periphyton community composition and availability of suspended Fine Particulate Organic Material (FPOM). Scrapers increase with increased diatom abundance and decrease as filamentous algae and aquatic mosses increase. However, filamentous algae and aquatic mosses provide good attachment sites for filtering collectors, and the organic enrichment often responsible for the filamentous algae can also provide FPOM that is utilized by the filterers.

Filtering collectors are also sensitive to toxicants bound to fine particles and should be the first group to decrease when exposed to steady sources of such toxicants. This situation is often associated with point-source discharges where certain toxicants adsorb readily to dissolved organic matter forming FPOM during flocculation. Toxicants thus become available to filterers via FPOM. A description of the Functional Feeding Group concept can be found in Cummins (1973) and Merritt and Cummins (1984). Most aquatic insects can be classified to Functional Feeding Group on the basis of morphological and behavioral features using Cummins and Wilzbach (1985).

Ratio of Shredders/Total

The percentage of the Shredder Functional Feeding Group to total number of organisms measures the relative abundance of organisms classified as shredders, which are sensitive to riparian zone impacts (Barbour et al. 1992).

METHODS OF ANALYSES

Ordination analysis

Because ordination analysis was so extensively used in this report, a discussion of its utility seems warranted. The technique compares the relative similarity of benthic invertebrate communities from all sites. The two dimensional plot allows spatial representation of communities whereby more similar communities are grouped close together while dissimilar ones are further apart. Thus a qualitative idea of how similar the fauna of one site is compared to the fauna of any other site is possible by assessing their relative distances apart on the graph (Gauch 1982).

Of importance is the pattern of sites on the ordination. Ordination is not a statistical test whereby we can evaluate and reject hypotheses, it is merely a tool which allows the reduction of the mass of data in a species-by-site matrix to more understandable and interpretable from. We used Detrended Correspondence analysis (DCA) by the computer program MultiVariate Statistical Package, MSVP, (Kovach 1993).

Boxplots

Boxplots (Fig. 1) are used in this report to display relations of metrics, either individually or combined into an index, under different circumstances. Boxplots provide a visual representation of several important features of a dataset: central tendency—the median value—and the variation of the data as the 10th, 25th, 75th, and 90th percentiles, as well as the skewness of the data and any outliers of data points. They are particularly useful in comparing multiple datasets. We have also used them as a basis for scoring metrics for inclusion in a biocriteria index.

Statistical Tests

Commonly used parametric tests, t-test, ANOVA, and correlations were used to examine differences in metrics spatially or temporally. Before testing, data were checked for normality using a normal probability plot (Systat 1990) or a Kolgomorov-Smirnow test (SAS). In some cases data were arc-sine or log transformed before analysis.

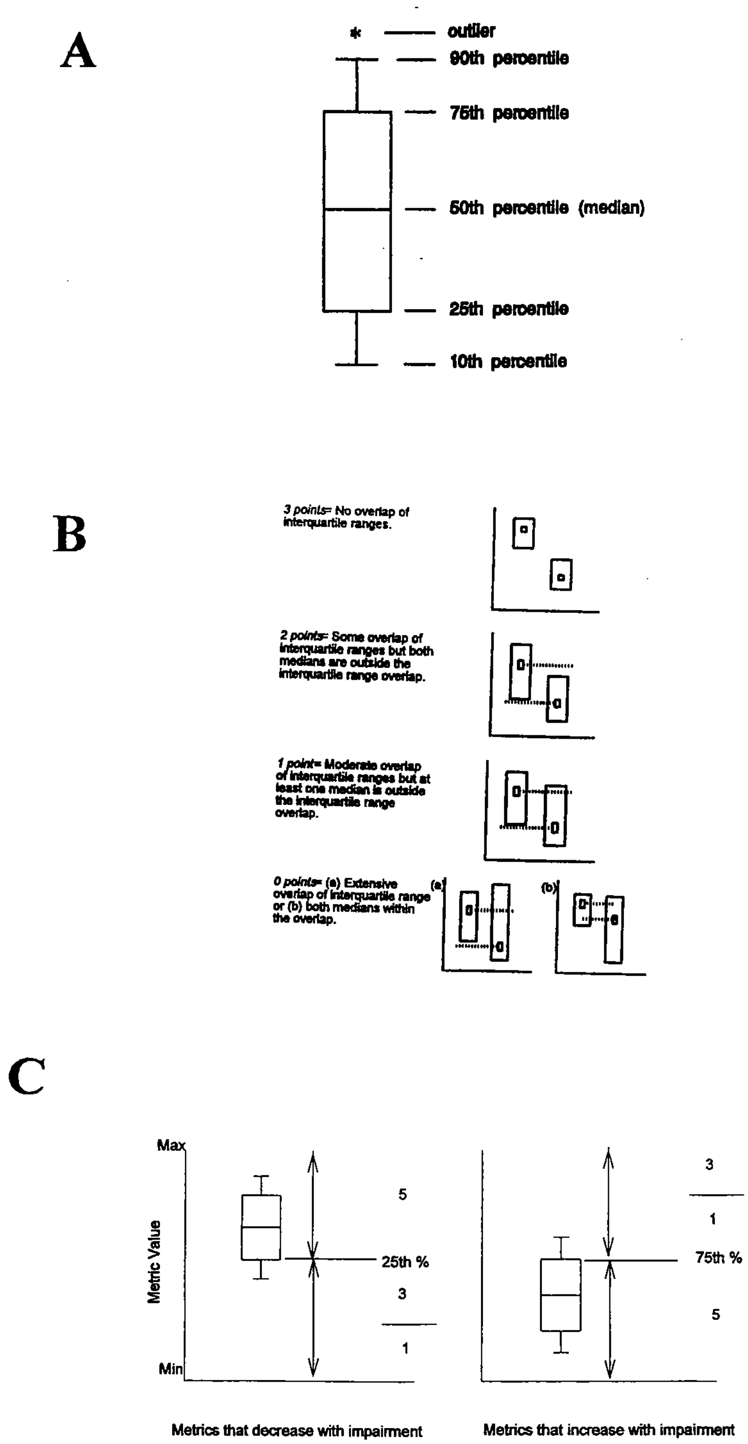


Fig. 1. A. Explanation of a boxplot; B. Scoring system used to evaluate sensitivity of metrics (Chapter 8) after Barbour et al 1996; C. An illustration of the metric scoring procedure used to develop the SCI index (Chapter 9).

Chapter 7

ANALYSIS OF REFERENCE STREAMS

Introduction

The goal of this chapter is to characterize reference stream benthic invertebrate communities for the major ecoregions of Missouri. The specific objectives are to: 1) document variation in benthic invertebrate community structure among three ecoregions of Missouri and within each region and 2) evaluate the performance of several indices, or metrics, in their ability to describe existing conditions.

Two surveys of all candidate reference streams were conducted: one in spring and one in fall of 1993. We attempted to minimize several sources of variation (see Chapters 3, 4, and 5). We sampled only in streams of a particular size, according to a strict protocol. We minimized temporal variation by sampling in as short a time period as possible and sampling south to north in spring and north to south in fall. Collected samples were processed and identified by the same personnel. Such restrictions on sampling design and methodologies gave us confidence that results would be due primarily to natural variation inherent in the invertebrate communities.

Data were analyzed in two stages. First, the similarity of invertebrate community structure at all sites was compared by ordination (Detrended Correspondence Analysis [DCA]) where a two-dimensional plot allows spatial representation of invertebrate communities whereby more similar communities are grouped close together while dissimilar ones are further apart (Gauch 1982). Thus a qualitative idea of how alike one site is compared to any other is possible.

The second stage of analyses evaluated the ability of indices or metrics to document patterns we observed on the ordination. For example, if the ordination showed a clear separation between communities from prairie streams and those from Ozark streams, we examined whether these differences were evident by any or all of our chosen metrics. Thus we were able to evaluate a suite of metrics to find those with low variation yet good discriminatory power.

In the spring of 1993, 45 reference streams (16 in the prairie region, 26 from the Ozark region, and 3 from the Mississippi Alluvial Plain—hereafter termed "lowland"—were sampled (Fig. 1). A replicate site was sampled on nine of the streams. All reference streams sampled in spring 1993 were sampled again in the fall, except Huffstetter Lateral Ditch, which was dry at the time. Eight of the streams sampled in fall had replicate sites. High water events during the summer of 1993 altered the physical nature of some sites so that some habitats were different between spring and fall.

All available habitat types found at each site were sampled. However, not all habitat types were present at each site (Table 1 spring; Table 2 fall). Generally in the prairie region cs flow (coarse substrate with flow), nonflow, rootmats, and fs flow (fine substrate with flow) were common while snags, leaf packs, and boulders were uncommon. In the Ozark streams the cs flow, nonflow, and rootmat habitats were common both seasons, while leaf packs were common in spring. In lowland streams only the habitat fs flow was present. Some of the analyses for spring 1993 were conducted on all available habitats, while

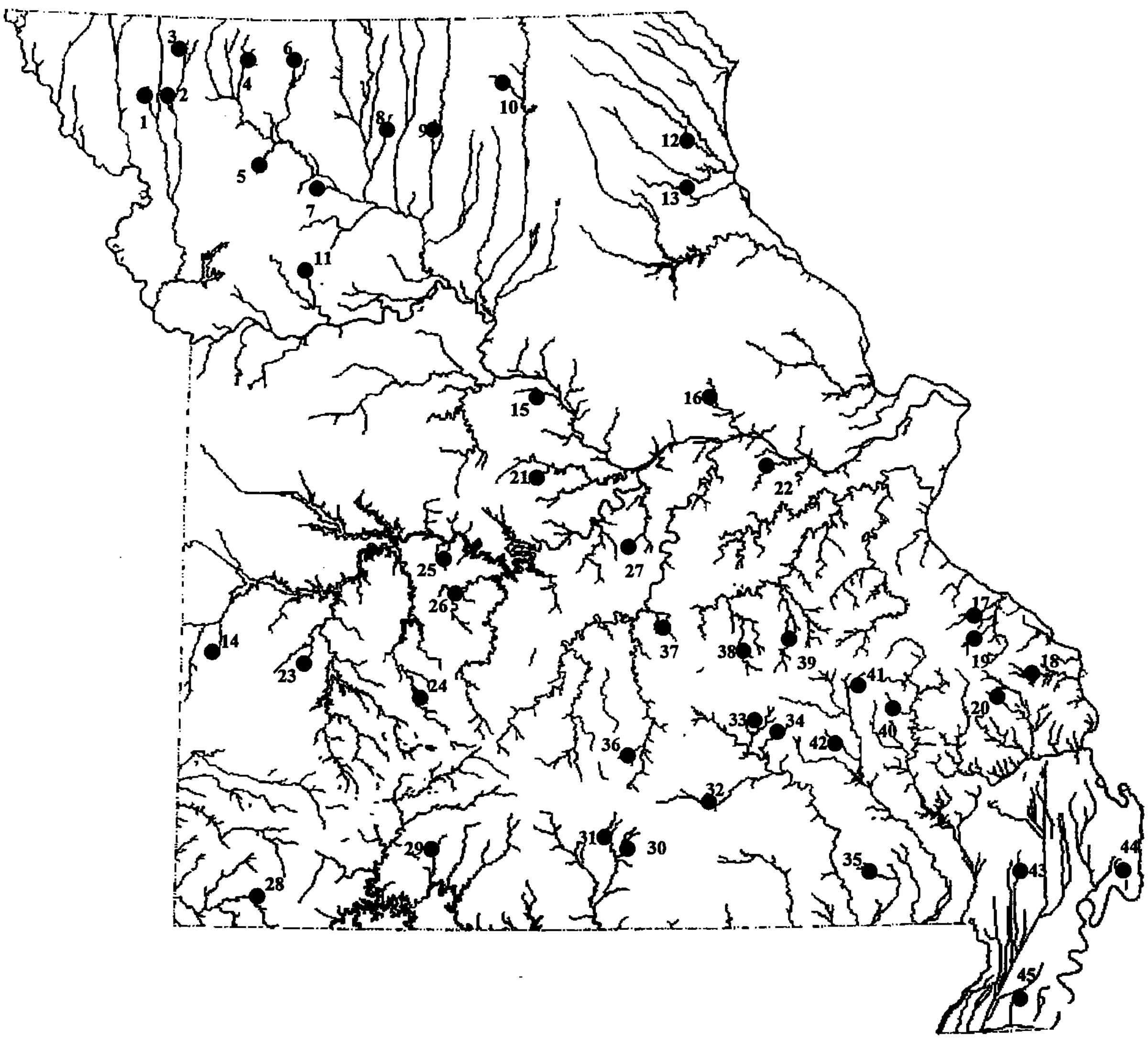


Fig. 1. Locations of all reference streams, 1993.

Table 1. Reference streams sampled during spring 1993 with a listing of all available habitats.

Stream	Str.No	Region	CS FLOW	NONFLOW	ROOT MAT	SNAG	FS FLOW	LEAF PACK	BOULDER
White Cloud	1	Prairie		1	1	1			
Long Br. Platt	2	Prairie	1		1		1		
Honey Cr.	3	Prairie	1	1	1				
E.Fk.Grand	4	Prairie	1		1		1		
Grindstone	5	Prairie	1		1		1		
W.Fk.Big	6	Prairie			1		1		
Marrowbone	7	Prairie	1	1	1		1		
No Cr.	8	Prairie		1	1				
W.Locust	9	Prairie	1	1	1	1	1	1	
Spring(Ada.)	10	Prairie		1	1	1	1		
E.F.Crooked	11	Prairie			1	1			
Mid.Fabius	12	Prairie	1		1		1		
North R.	13	Prairie	1	1	1		1		
Ltl.Dry Wood	14	Prairie		1			1	1	
Petite Saline	15	Prairie	1	1	1	1			
Loutre	16	Prairie	1	1	1	1			
SUM			**10**	**10**	**15**	**6**	**10**	**2**	**0**
R.Aux Vasse	17	Ozark	1	1	1			1	
Apple	18	Ozark	1	1	1			1	
Saline	19	Ozark	1	1	1	1		1	
Whitewater	20	Ozark	1	1				1	
Burris	21	Ozark	1	1		1		1	
Boeuf	22	Ozark	1	1	1	1		1	
Cedar	23	Ozark	1	1	1				
Pomme	24	Ozark	1	1	1			1	
Deer Cr.	25	Ozark	1	1	1			1	
Ltl.Niangua	26	Ozark	1	1	1	1			
Ltl.Maries	27	Ozark	1						
Big Sugar	28	Ozark	1	1		1		1	
Bull	29	Ozark	1	1	1				
Spring (Doug.	30	Ozark	1	1	1			1	
North fork	31	Ozark	1		1			1	
Jacks	32	Ozark	1	1				1	
Sinking(Sha.)	33	Ozark	1	1	1	1		1	
Big Cr.	34	Ozark	1	1		1			
Ltl. Black	35	Ozark	1	1	1	1			
W. Piney	36	Ozark	1	1		1		1	
Ltl. Piney	37	Ozark	1	1	1				
Meramac	38	Ozark	1	1	1			1	
Huzzah	39	Ozark	1	1	1			1	
Marble	40	Ozark	1	1	1			1	1
E. Fk. Black	41	Ozark	1	1				1	1
Sinking(Rey.)	42	Ozark	1	1	1	1		1	
SUM			**26**	**24**	**18**	**10**	**0**	**19**	**2**
Huffstetter	43	Lowland					1		
Ash Slough	44	Lowland					1		
Maple Slough	45	Lowland					1		
SUM			**0**	**0**	**0**	**0**	**3**	**0**	**0**

Table 2. Reference streams sampled during fall 1993 with a listing of all available habitats.

Stream	Str.No	Region	CS FLOW	NONFLOW	ROOT MAT	SNAG	FS FLOW	LEAF PACK	VEG
White Cloud	1	Prairie		1	1	1	1		
Long Br. Platt	2	Prairie		1	1		1		
Honey Cr.	3	Prairie	1	1	1				
E.Fk.Grand	4	Prairie	1		1	1	1		
Grindstone	5	Prairie	1	1	1	1	1		
W.Fk.Big	6	Prairie		1	1	1	1	1	
Marrowbone	7	Prairie		1			1	1	
No Cr.	8	Prairie	1	1	1				
W.Locust	9	Prairie	1	1	1	1			
Spring(Ada.)	10	Prairie			1	1	1		
E.F.Crooked	11	Prairie		1	1	1			
Mid.Fabius	12	Prairie	1	1	1		1		
North R.	13	Prairie	1	1	1		1		
Ltl.Dry Wood	14	Prairie			1		1		
Petite Saline	15	Prairie	1	1	1	1			
Loutre	16	Prairie	1	1	1	1			
SUM			**9**	**13**	**15**	**9**	**10**	**2**	**0**
R.Aux Vasse	17	Ozark	1	1	1				1
Apple	18	Ozark	1	1	1				
Saline	19	Ozark	1	1	1				
Whitewater	20	Ozark	1	1					
Burris	21	Ozark	1	1	1	1			
Boeuf	22	Ozark	1	1	1	1			
Cedar	23	Ozark	1	1	1				
Pomme	24	Ozark	1	1	1				
Deer Cr.	25	Ozark	1	1	1	1			
Ltl.Niangua	26	Ozark	1	1	1				
Ltl.Maries	27	Ozark	1	1	1				1
Big Sugar	28	Ozark	1	1	1				
Bull	29	Ozark	1	1	1				
Spring (Doug.	30	Ozark	1	1	1				
North fork	31	Ozark	1	1	1				
Jacks	32	Ozark	1	1					1
Sinking(Sha.)	33	Ozark	1	1	1				
Big Cr.	34	Ozark	1	1	1				
Ltl. Black	35	Ozark	1	1	1	1			1
W. Piney	36	Ozark	1	1	1				
Ltl. Piney	37	Ozark	1	1	1				
Meramac	38	Ozark	1	1	1				1
Huzzah	39	Ozark	1	1	1				
Marble	40	Ozark	1	1					1
E. Fk. Black	41	Ozark	1	1					1
Sinking(Rey.)	42	Ozark	1	1					1
SUM			**26**	**26**	**21**	**4**	**0**	**0**	**8**
Huffstetter	43	Lowland							
Ash Slough	44	Lowland					1		
Maple Slough	45	Lowland			1	1	1		
SUM			**0**	**0**	**1**	**1**	**2**	**0**	**0**

other analyses for spring data and all analyses for fall data were conducted on either single habitat data or "multihabitat" data. Multihabitat analyses consisted of using a dataset where organisms from three habitats in common (cs flow, nonflow, and rootmats) were used. Reasons for using data from various habitat combination are explained in the text.

ANALYSES

The Benthic Fauna

Over 280 taxa were collected during the two sampling periods (Table 3).

Nutrients and Habitat Scores

Lowland streams from the Mississippi Alluvial Plain ecoregion had the highest total phosphorus (TP) and lowest habitat scores of any region (Table 4). Prairie sites generally had lower habitat condition scores but higher nutrient concentrations than Ozark stream sites. Some seasonal differences were noted but the same among region differences existed.

Benthic Invertebrate Community Structure (spring—all habitats)

When all habitats from each site were used in the ordination there was a good separation of streams based on geography (Fig. 2), where Ozark, prairie, and lowland sites were clearly separated. Lowland sites grouped by themselves while there was some overlap of prairie and Ozark sites. At the area of overlap, Ozark streams are considered transitional in geography (as Ozark Border Stream 21, see Fig. 1), while prairie overlap streams are from the northeast part of the state (streams 12 and 13, Fig. 1). These results confirm there is a definite regionalized fauna, and that the fauna within a region is fairly homogeneous. Or to put it another way, among region differences in community structure are greater than within region differences.

There is also some evidence of subregionalization. For example, prairie sites in the northeastern sector of the state group together and are more similar to Ozark sites than are the other Prairie sites. They overlap with a group of sites from the west-central region of the state. Further work with more streams may be able to divide the prairie into northeast prairie and northwest prairie and the Ozarks into southeast and west-central Ozarks, but for several important reasons we decided to develop a biomonitoring framework using three ecoregions.

Comparison of Metrics Among Regions (spring—all habitats)

The spring 1993 sampling was our first opportunity to evaluate some common indices, or metrics, for Missouri streams. We chose an initial suite of 11 metrics (see Chapter 6 for rationale). Table 5 lists metric values for all sites. Statistical analysis by ANOVA indicates 8 metrics were significantly different among all three regions ($P < 0.001$; Fig. 3). Nonsignificant metrics were those that were ratios of various taxa or functional groups, except for one case.

Variation of the metrics was examined by plotting the coefficient of variation (CV) of each metric by region (Fig. 4). Those metrics employing ratios of one taxa to another or one functional group to another had the greatest variation. Intermediate in variation was Simpson's Diversity Index and % Dominant taxon. Low variation was shown by metrics Total taxa, Family, EPT (except lowland), Biotic Index (BI), and Shannon's Diversity Index. From these results we concluded that the four metrics developed by using ratios of one

Table 3. Biological Criteria Project macroinvertebrate taxa list for reference streams, spring (S) and fall (F) 1993.

Arthropoda

Arachnida
- Acarina
  - *Lebertia* (S)
  - *Hydrachna* (S, F)

Crustacea
- Isopoda
  - Asellidae
    - *Caecidotea* (S, F)
    - *Lirceus* (S, F)
- Amphipoda
  - Crangonyctidae
    - *Crangonyx* (S)
    - *Synurella* (S, F)
  - Gammaridae
    - *Allocrangonyx* (S)
    - *Gammarus* (S, F)
  - Talitiridae
    - *Hyalella azteca* (S, F)
- Decapoda
  - Cambaridae
    - *Cambarus* (S)
    - *Orconectes* (S, F)
  - Palaemonidae
    - *Palaemontes*
      - *Palaemontes kadiakensis* (S)

Insecta
- Ephemeroptera
  - Baetidae
    - *Acentrella* (S, F)
    - *Baetis* (S, F)
    - *Procloeon* (F)
  - Baetiscidae
    - *Baetisca*
    - *Baetisca lacustris* (S, F)
    - *Baetisca obesa* (S)
  - Caenidae
    - *Brachycerus* (F)
    - *Caenis*
      - *Caenis amica* (S)
      - *Caenis anceps* (F)
      - *Caenis hilaris* (F)
      - *Caenis latipennis* (S, F)
      - *Caenis punctata* (S, F)
  - Ephemeridae
    - *Ephemera* (S, F)
    - *Hexagenia* (S, F)

Table 3 (continued).

Epherellidae
- *Ephemerella*
  - *Ephemerella invaria* (S)
  - *Ephemerella* sp. (S)
- *Eurylophella*
  - *Eurylophella bicolor* (S)
  - *Eurylophella temporalis* (S)
  - *Eurylophella lutuleta* (S)
- *Serratella* (F)

Heptageniidae
- *Epeorus* (S)
- *Heptagenia*
  - *Heptagenia diabasia* (S, F)
- *Leucrocuta* (S,F)
- *Stenacron* (S, F)
- *Stenonema*
  - *Stenonema bednariki* (S)
  - *Stenonema exiguum* (F)
  - *Stenonema femoratum* (S, F)
  - *Stenonema integrum* (F)
  - *Stenonema mediopunctatum* (S, F)
  - *Stenonema pulchellum* (S, F)
  - *Stenonema terminatum* (S, F)
  - *Stenonema vicarium* (S, F)

Leptophlebiidae
- *Choroterpes* (F)
- *Leptophlebia* (S)
- *Paraleptophlebia* (S, F)

Oligoneuriidae
- *Isonychia*
  - *Isonychia bicolor* (S, F)
  - *Isonychia rufa* (S, F)

Potamanthidae
- *Anthopotamus*
  - *Anthopotamus myops* (S, F)

Siphlonuridae
- *Siphlonurus* (S)

Tricorythidae
- *Tricorythodes* (S, F)

Odonata
- Calopterygidae
  - *Calopteryx* (S, F)
  - *Hetaerina* (F)
- Coenagrionidae
  - *Argia* (S, F)
  - *Chromagrion* (S)
  - *Enallagma* (S, F)
- Corduliidae
  - *Neurocordulia* (S, F)
  - *Tetragoneuria* (S, F)

Table 3 (continued).

Libellulidae
- *Erythemis* (S, F)
- *Libellula* (S)
- *Pachydiplax* (S)
- *Perithemis* (F)

Aeshnidae
- *Aeshna* (S, F)
- *Boyeria* (S, F)
- *Nasiaeschna*
  - *Nasiaeschna pentacantha* (F)

Gomphidae
- *Arigomphus* (S)
- *Dromogomphus* (S, F)
- *Erpetogomphus* (F)
- *Gomphus* (S, F)
- *Hagenius*
- *Hagenius brevistylus* (S, F)
- *Ophiogomphus* (S)
- *Progomphus*
  - *Progomphus obscurus* (S, F)
- *Stylogomphus*
  - *Stylogomphus albistylus* (S, F)

Macromiidae
- *Didymops* (S, F)
- *Macromia* (S, F)

Plecoptera

Capniidae
- *Allocapnia* (S)
- *Paracapnia* (S)

Leuctridae
- *Leuctra* (S, F)
- *Zealeuctra* (S, F)

Nemouriidae
- *Amphinemura* (S)
- *Prostoia*
  - *Prostoia completa* (S)

Perlidae
- *Acroneuria*
  - *Acroneuria frisoni* (S, F)
- *Attaneuria* (S)
- *Neoperla*
  - *Neoperla falayah* (S, F)
  - *Neoperla harpi* (S, F)
  - *Neoperla osage* (F)
- *Perlinella*
  - *Perlinella drymo* (S)
  - *Perlinella ephyre* (S, F)
- *Perlesta*
  - *Perlesta decipiens* (S)

Table 3 (continued).

- Perlodidae
  - *Clioperla*
    - *Clioperla clio* (S)
  - *Isoperla*
    - *Isoperla decepta* (S)
    - *Isoperla dicala* (S)
    - *Isoperla namata* (S)
    - *Isoperla signata* (S)
    - *Isoperla mohri* (S)
  - *Hydroperla*
    - *Hydroperla crosbyi* (S)
- Pteronarcyidae
  - *Pteronarcys*
    - *Pteronarcys pictetii* (S, F)
- Taeniopterygidae
  - *Strophopteryx*
    - *Strophopteryx arkansae* (S)
    - *Strophopteryx fasciata* (S)
  - *Taeniopteryx*
    - *Taeniopteryx burksi* (S)

Hemiptera
- Belostomatidae
  - *Belostoma* (S, F)
- Corixidae
  - *Trichocorixa* (S, F)
- Gerridae
  - *Gerris* (S, F)
  - *Metrobates* (F)
  - *Rheumatobates* (F)
  - *Trepobates* (F)
- Hydrometridae
  - *Hydrometra* (F)
- Nepidae
  - *Ranatra*
    - *Ranatra nigra* (S, F)
- Pleidae
  - *Neoplea* (F)
- Veliidae
  - *Microvelia* (S, F)
  - *Rhagovelia* (F)

Megaloptera
- Corydalidae
  - *Chauliodes* (S)
  - *Corydalus* (S, F)
  - *Nigronia* (S, F)
- Sialidae
  - *Sialis* (S, F)

Coleoptera
- Anthicidae (S)

Table 3 (continued).

- Dryopidae
    - *Helichus* (S, F)
- Dytiscidae
    - *Hydaticus* (S, F)
    - *Hydroporus* (S,F)
    - *Hydrovatus* (S, F)
    - *Laccophilus* (S, F)
    - *Uvarus* (S)
- Elmidae
    - *Ancronyx* (S, F)
    - *Dubiraphia* (S, F)
    - *Macronychus* (S, F)
    - *Optioservus* (S, F)
    - *Stenelmis* (S, F)
- Georyssidae
    - *Georyssus* (S)
- Gyrinidae
    - *Dinetus* (S, F)
    - *Gyrinus* (S, F)
- Haliplidae
    - *Haliplus*
    - *Peltodytes* (S, F)
- Heteroceridae (S, F)
- Hydrophilidae
    - *Berosus* (S, F)
    - *Enochrus* (F)
    - *Helochares* (F)
- Helophoridae
    - *Heliophorus* (F)
- Hydrochidae
    - *Hydrochus* (S, F)
    - *Paracymus* (S)
    - *Tropisternus* (S, F)
- Lampyridae (S)
- Lutrochidae
    - *Lutrochus* (S)
- Psephenidae
    - *Ectopria* (S, F)
    - *Psephenus* (S, F)
- Salpingidae (F)
- Scirtidae
    - *Scirtes* (S, F)
- Staphylinidae
    - *Carpelimus* (S, F)
    - *Stenus* (F)
    - *Thinopinus* (F)

Trichoptera

- Brachycentridae
    - *Brachycentrus* (S)
    - *Microsema* (S, F)

Table 3 (continued).

Glossosomatidae
- *Agapetus* (S, F)
- *Glossosoma* (F)

Helicopsychidae
- *Helicopsyche* (S, F)

Hydropsychidae
- *Ceratopsyche* (S, F)
- *Cheumatopsyche* (S, F)

Hydroptilidae
- *Hydroptila* (S, F)
- *Ochrotrichia* (S)
- *Orthotrichia* (S)
- *Oxyethira* (S, F)

Leptoceridae
- *Ceraclea* (F)
- *Mystacides* (F)
- *Nectopsyche* (S, F)
- *Oecetis* (S, F)
- *Triaenodes* (F)

Lepidostomatidae
- *Lepidostoma* (S)

Limnephilidae
- *Ironoquia* (S)
- *Neophylax* (S)
- *Pycnopsyche* (S)

Odontoceridae
- *Marilia* (F)
- *Psilotreta* (F)

Philopotamidae
- *Chimarra* (S, F)

Phryganeidae
- *Ptilostomis* (S)

Polycentropodidae
- *Cernotina* (S, F)
- *Neureclipsis* (F)
- *Paranyctiophylax* (S)
- *Polycentropus* (F)

Psychomyiidae
- *Lype* (F)
- *Psychomyia* (S, F)

Rhyacophilidae
- *Rhyacophila* (S)

Lepidoptera
- Noctuidae
  - *Bellura* (S)
  - *Simyra* (F)
- Pyralidae
  - *Paraponyx* (F)
  - *Petrophila* (S, F)
- Unknown - EFBR (S)

Table 3 (continued).

- Diptera
  - Athericidae
    - *Atherix* (S)
  - Ceratopogonidae
    - All (except *Atrichopogon*) (S, F)
    - *Atrichopogon* (S, F)
  - Chironomidae
    - Chironominae
      - Chironomini
        - *Acalcarella* (S)
        - *Axarus* (S, F)
        - *Cladopelma* (F)
        - *Chironomus* (S, F)
        - *Cryptochironomus* (S, F)
        - *Cryptotendipes* (F)
        - *Demicryptochironmus* (F)
        - *Dicrotendipes* (S, F)
        - *Endochironomus* (S)
        - *Glypotendipes* (S, F)
        - *Hyporhygma* (S)
        - *Lipiniella* (S)
        - *Microtendipes* (S, F)
        - *Parachironomus* (S)
        - *Paracladopelma* (F)
        - *Paralauterborniella* (F)
        - *Paratendipes* (S, F)
        - *Phaenopsectra* (S, F)
        - *Polypedilum* (S, F)
        - *Saetheria* (S, F)
        - *Stenochironomus* (S, F)
        - *Stictochironomus* (S, F)
        - *Tribelos* (S, F)
      - Psuedochironmini
        - *Psuedochironomus* (S, F)
      - Tanytarsini
        - *Constempellina* (S)
        - *Cladotanytarsus* (Cladotanytarsus) (S, F)
        - *Cladotanytarsus* (Lienziella) (S, F)
        - *Micropsectra/Tanytarsus* (S, F)
        - *Paratanytarsus* (S, F)
        - *Rheotanytarsus* (S, F)
        - *Stempellinella* (S, F)
        - *Sublettea* (S, F)
        - *Tanytarsus* (S, F)
    - Diamesinae
      - *Diamesa* (S)
      - *Potthastia* (S)
      - *Psuedodiamesa* (S)
      - *Sympotthastia* (S)

- Orthocladiinae
    - *Brillia* (S, F)
    - *Cardocladius* (F)
    - *Corynoneura* (S, F)
    - *Cricotopus* (S)
    - *Cricotopus/Orthocladius* (S, F)
    - *Diplocladius* (S)
    - *Doncricotopus* (S)
    - *Eukiefferiella* (S, F)
    - *Hydrobaenus* (S)
    - *Nanocladius* (S, F)
    - *Oliveridia* (S)
    - *Orthocladius* (Euorthocladius) (S)
    - *Parametriocnemus* (S)
    - *Parakiefferiella* (S)
    - *Paraphaenocladius* (S)
    - *Parorthocladius* (S)
    - *Psectrocladius* (S)
    - *Psuedorthocladius* (S)
    - *Rheocricotopus* (S, F)
    - *Smittia* (S)
    - *Symposiocladius* (S)
    - *Thienemanniella* (S, F)
    - Unknown A (S)
    - *Xylotopus* (S)
- Prodiamesinae
    - *Monodiamesa* (S, F)
- Tanypodinae
    - *Ablabesmyia* (F)
    - *Clinotanypus* (S)
    - *Djalmabatista* (S)
    - *Krenopelopia* (S, F)
    - *Larsia* (S, F)
    - *Nilotanypus* (S, F)
    - *Procladius* (S, F)
    - *Tanypus* (S, F)
    - *Thienemannimyia* gr. (S)

Chaoboridae
- *Chaoborus* (F)

Culicidae
- *Anopheles* (F)

Dixidae
- *Dixa* (F)
- *Dixella* (S, F)

Dolichopodidae (S, F)

Empididae
- *Chelifera* (S, F)
- *Clinocera* (S,F)
- *Hemerodromia* (S, F)

Ephydridae (F)

Muscidae (S, F)

Table 3 (continued).

Psychodidae
- *Pericoma* (S)

Simulidae
- *Cnephia* (S)
- *Prosimulium* (S, F)
- *Simulium* (S, F)

Stratiomyiidae
- *Myxosargus* (S)
- *Nemotelus* (S)

Tabanidae
- *Chrysops* (S, F)
- *Silvius* (S, F)
- *Tabanus* (S, F)

Tanyderidae
- *Protoplasma*
  - *Protoplasma fitchii* (S)

Tipulidae
- *Antocha* (S)
- *Dicranota* (S)
- *Hexatoma* (S, F)
- *Limonia* (S)
- *Limnophila* (S, F)
- *Rhabdomastix* (F)
- *Tipula* (S, F)

Hymenoptera
- Braconidae (F)
- Scelionidae (S)

Non-Arthropods

Annelida
- Hirudinea (S, F)
- Oligochaeta (S, F)

Gastropoda
- Ancylidae
  - *Ferrissia* (S, F)
  - *Laevapex* (S)
- Hydrobiidae (S, F)
- Lymnaeidae
  - *Fossaria* (S, F)
  - *Pseudosuccinea*
    - *Pseudosuccinea columella* (S)
- Physidae
  - *Physella* (S, F)
- Planorbidae (S, F)
- Pleuroceridae
  - *Elimia* (S, F)
  - *Pleurocera* (S, F)

Table 3 (continued).

Nematomorpha
Gordiidae
Gordius (S)
Turbellaria
Planariidae (S, F)

Table 4. Nutrient Chemistries and Habitat Scores for Reference Streams, 1993

Stream	Str.no.	Region	Spring			Fall		
			TN (mg/l)	TP (ug/L)	Score	TN (mg/l)	TP (ug/L)	Score
White Cloud	1	Prairie	3.33	180	63	0.97	164	59
Long Br. Platte	2	Prairie	2.82	225	84	0.80	172	56
Honey Cr.	3	Prairie	1.00	160	103	0.80	150	114
E.Fk.Grand	4	Prairie	1.76	150	86	0.88	98	86
Grindstone	5	Prairie	1.20	110	105	1.02	164	93
W.Fk.Big	6	Prairie	1.00	169	57	0.62	98	60
Marrowbone	7	Prairie	0.94	74	65	0.90	91	54
No Cr.	8	Prairie	0.98	144	50	1.22	163	72
W.Locust	9	Prairie	0.47	44	76	0.62	83	93
Spring(Ada.)	10	Prairie	0.30	66	66	0.28	50	60
E.F.Crooked	11	Prairie	0.74	86	80	0.62	25	104
Mid.Fabius	12	Prairie	0.93	129	79	0.62	80	92
North R.	13	Prairie	1.00	92	67	0.56	68	48
Ltl.Dry Wood	14	Prairie	0.26	38	77	1.08	184	89
Petite Saline	15	Prairie	2.30	110	99	1.53	146	113
Loutre	16	Prairie	1.10	98	116	0.75	102	117
R.Aux Vasse	17	Ozark	0.18	14	119	0.26	22	92
Apple	18	Ozark	0.82	17	100	0.24	22	93
Saline	19	Ozark	0.41	9	129	0.30	15	112
Whitewater	20	Ozark	0.32	6	142	0.18	8	112
Burris	21	Ozark	1.80	152	133	0.34	66	106
Boeuf	22	Ozark	0.84	16	130	0.56	34	118
Cedar	23	Ozark	0.50	19	135	1.03	70	106
Pomme	24	Ozark	0.50	28	140	0.98	88	127
Deer Cr.	25	Ozark	0.10	6	148	0.28	20	127
Ltl.Niangua	26	Ozark	0.25	30	148	0.34	31	132
Ltl.Maries	27	Ozark	0.70	88	118	0.66	42	131
Big Sugar	28	Ozark	2.06	20	141	2.10	26	128
Bull	29	Ozark	0.60	4	127	0.82	6	116
Spring (Doug.)	30	Ozark	0.18	6	152	0.32	10	145
North fork	31	Ozark	0.56	4	145	0.47	16	138
Jacks	32	Ozark	0.19	4	163	0.18	6	147
Sinking(Sha.)	33	Ozark	0.10	3	149	0.08	3	132
Big Cr.	34	Ozark	0.13	4	150	0.14	4	138
Ltl. Black	35	Ozark	0.08	8	135	0.18	12	137
W. Piney	36	Ozark	0.78	9	148	1.83	117	144
Ltl. Piney	37	Ozark	0.58	13	146	0.65	12	140
Meramac	38	Ozark	0.19	4	154	0.12	8	110
Huzzah	39	Ozark	0.22	3	148	0.12	3	123
Marble	40	Ozark	0.06	4	159	0.43	10	133
E. Fk. Black	41	Ozark	0.06	4	138	0.08	5	136
Sinking(Rey.)	42	Ozark	0.19	5	134	0.15	2	126
Huffstetter	43	Lowland	0.92	146	23			
Ash Slough	44	Lowland	0.28	170	21	0.45	148	37
Maple Slough	45	Lowland	0.28	186	47	0.24	214	57

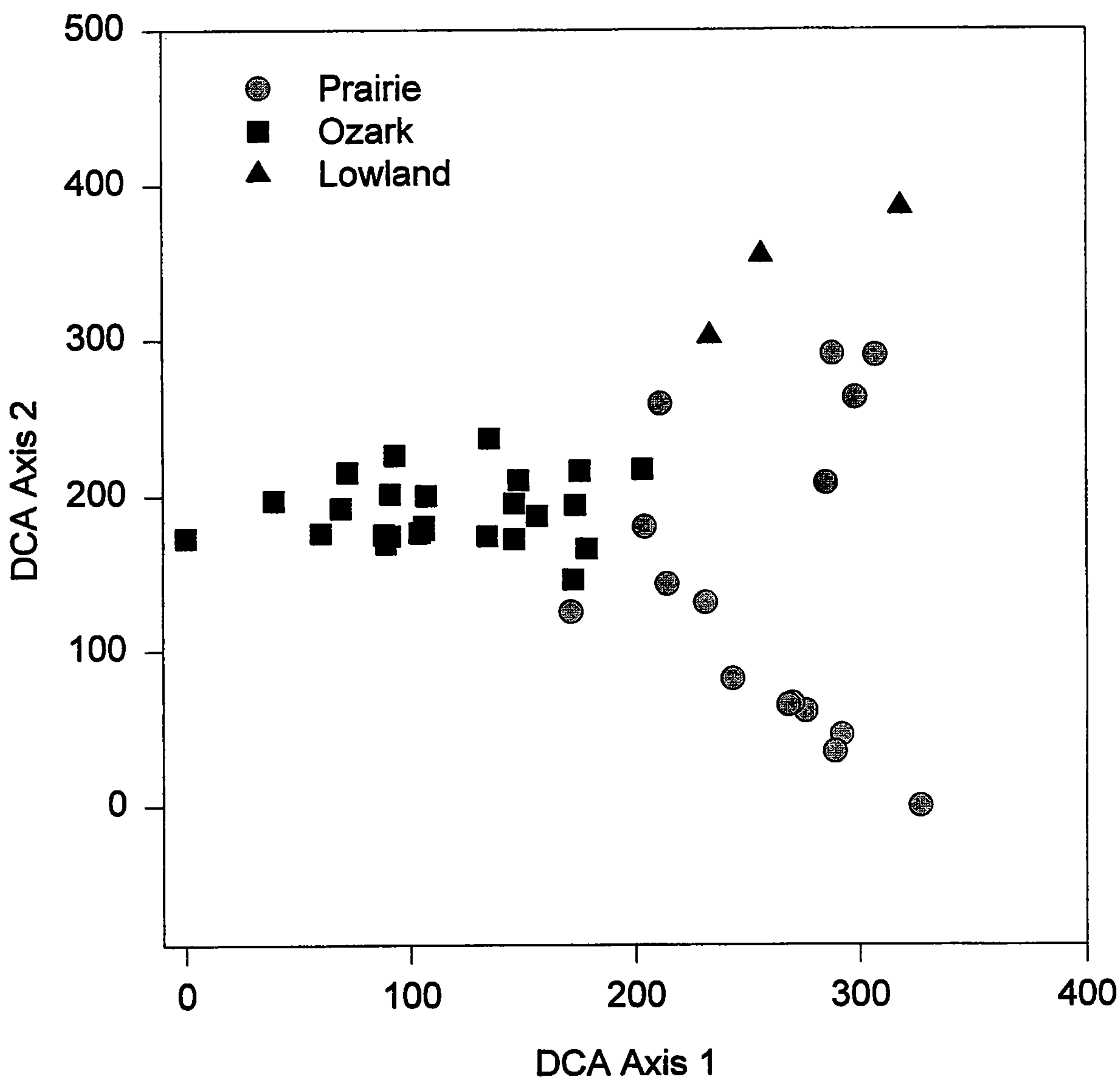


Fig. 2. Ordination of invertebrate communities from all reference sites, spring 1993, all habitats.

Table 5. Calculated metrics for each reference stream in spring of 1993. Analysis includes all habitats present at each site.

Stream	Str.No	Region	Hab.No	Taxa	Family	EPT	EPT/Chir.	Hydr./Trich	Dominant	Biotic Ind	Shannon	Simpson	Shred/Tot	Scrap/Filt
White Cloud	1	Prairie	3	31	18	4	2.63	1.00	39.5	7.3	2.22	0.21	0.04	7.32
Long Br. Platt	2	Prairie	3	31	16	4	12.35	1.00	74.7	7.8	1.14	0.57	0.02	2.29
Honey Cr.	3	Prairie	3	22	13	5	6.45	1.00	26.6	6.1	2.19	0.17	0.02	11.66
E.Fk.Grand	4	Prairie	3	26	14	5	2.76	1.00	28.5	6.9	2.54	0.13	0.08	3.13
Grindstone	5	Prairie	3	33	20	11	1.49	0.70	12.4	6.3	2.97	0.07	0.16	1.47
W.Fk.Big	6	Prairie	2	25	13	8	1.92	1.00	36.3	6.9	2.41	0.16	0.12	3.47
Marrowbone	7	Prairie	4	50	28	15	4.74	0.69	56.0	7.6	1.81	0.34	0.01	2.02
No Cr.	8	Prairie	2	21	14	7	1.07	0.37	35.9	7.1	2.23	0.18	0.06	0.10
W.Locust	9	Prairie	6	40	18	9	0.27	0.55	51.8	6.6	1.87	0.31	0.19	0.06
Spring(Ada.)	10	Prairie	4	43	23	10	0.45	0.21	47.2	7.1	2.07	0.27	0.54	1.80
E.F.Crooked	11	Prairie	2	35	22	10	1.25	0.64	20.8	6.7	2.85	0.09	0.12	1.70
Mid.Fabius	12	Prairie	3	35	23	10	5.45	1.00	26.4	5.9	2.71	0.12	0.07	7.40
North R.	13	Prairie	4	51	26	11	0.71	0.67	25.3	6.6	2.88	0.10	0.10	0.57
Ltl.Dry Wood	14	Prairie	3	44	19	12	0.20	0.53	56.5	6.5	1.94	0.34	0.16	0.04
Petite Saline	15	Prairie	4	35	21	6	0.16	0.64	46.5	6.9	1.86	0.27	0.19	0.02
Loutre	16	Prairie	4	44	28	4	0.20	*	33.5	7.5	2.51	0.17	0.33	11.77
R.Aux Vasse	17	Ozark	4	67	33	23	0.57	0.18	25.3	6.2	3.25	0.09	0.33	1.93
Apple	18	Ozark	4	49	26	12	0.81	0.88	20.9	6.6	2.89	0.10	0.30	6.64
Saline	19	Ozark	5	62	31	23	0.23	0.62	32.8	5.9	2.53	0.16	0.35	0.37
Whitewater	20	Ozark	3	50	28	17	0.48	0.57	26.5	5.6	2.63	0.12	0.19	3.13
Burris	21	Ozark	4	41	24	12	0.34	0.40	29.5	7.0	2.56	0.14	0.34	2.55
Boeuf	22	Ozark	5	43	26	8	0.09	*	43.2	6.5	2.29	0.22	0.50	1.99
Cedar	23	Ozark	3	47	26	18	1.26	0.26	15.8	5.9	3.16	0.07	0.14	2.18
Pomme	24	Ozark	4	57	35	16	0.75	0.71	14.1	5.7	3.15	0.07	0.18	0.50
Deer Cr.	25	Ozark	4	59	36	20	0.88	0.01	16.7	5.6	3.18	0.07	0.23	1.96
Ltl.Niangua	26	Ozark	4	61	32	16	0.23	0.37	19.9	6.7	3.06	0.09	0.25	1.22
Big Sugar	28	Ozark	4	61	27	26	3.73	0.86	20.9	5.0	2.90	0.09	0.04	21.72
Bull	29	Ozark	3	48	25	18	1.71	0.30	15.6	5.1	3.22	0.06	0.11	5.92
Spring (Doug.	30	Ozark	4	65	30	19	0.53	0.68	19.3	5.3	3.42	0.06	0.26	1.88
North fork	31	Ozark	3	54	24	19	1.59	1.00	11.3	4.7	3.47	0.04	0.16	2.46
Jacks	32	Ozark	3	53	26	18	1.61	0.60	14.4	4.6	3.18	0.06	0.20	2.21
Sinking(Sha.)	33	Ozark	5	59	28	18	0.97	1.00	11.3	4.5	3.34	0.05	0.14	6.12
Big Cr.	34	Ozark	3	46	21	13	0.50	0.64	18.7	5.4	3.00	0.09	0.07	21.90
Ltl. Black	35	Ozark	4	60	32	18	1.64	*	21.1	5.6	3.06	0.09	0.17	1.44
W. Piney	36	Ozark	4	51	32	21	1.46	0.50	22.7	4.7	3.06	0.09	0.19	1.25
Ltl. Piney	37	Ozark	3	51	33	22	0.60	0.70	45.0	5.9	2.49	0.22	0.48	0.88
Meramac	38	Ozark	4	67	38	23	0.90	0.47	26.2	5.0	3.19	0.10	0.17	2.46
Huzzah	39	Ozark	4	51	29	23	1.88	0.25	19.0	3.9	2.79	0.10	0.11	5.15
Marble	40	Ozark	5	68	38	25	1.61	0.77	30.7	4.5	2.97	0.12	0.11	0.61
E. Fk. Black	41	Ozark	4	52	32	24	4.01	0.20	31.9	3.9	2.93	0.12	0.14	0.52
Sinking(Rey.)	42	Ozark	5	59	33	20	0.63	0.25	33.9	4.3	2.74	0.15	0.21	1.79
Huffstetter	43	Lowland	1	13	7	0	0.00	*	68.0	8.5	1.19	0.49	0.05	0.00
Ash Slough	44	Lowland	1	14	8	1	0.06	*	46.7	7.7	1.87	0.26	0.17	1.00
Maple Slough	45	Lowland	1	13	10	3	1.60	*	54.5	7.9	1.49	0.35	0.04	10.00

* where Trich. were 0.

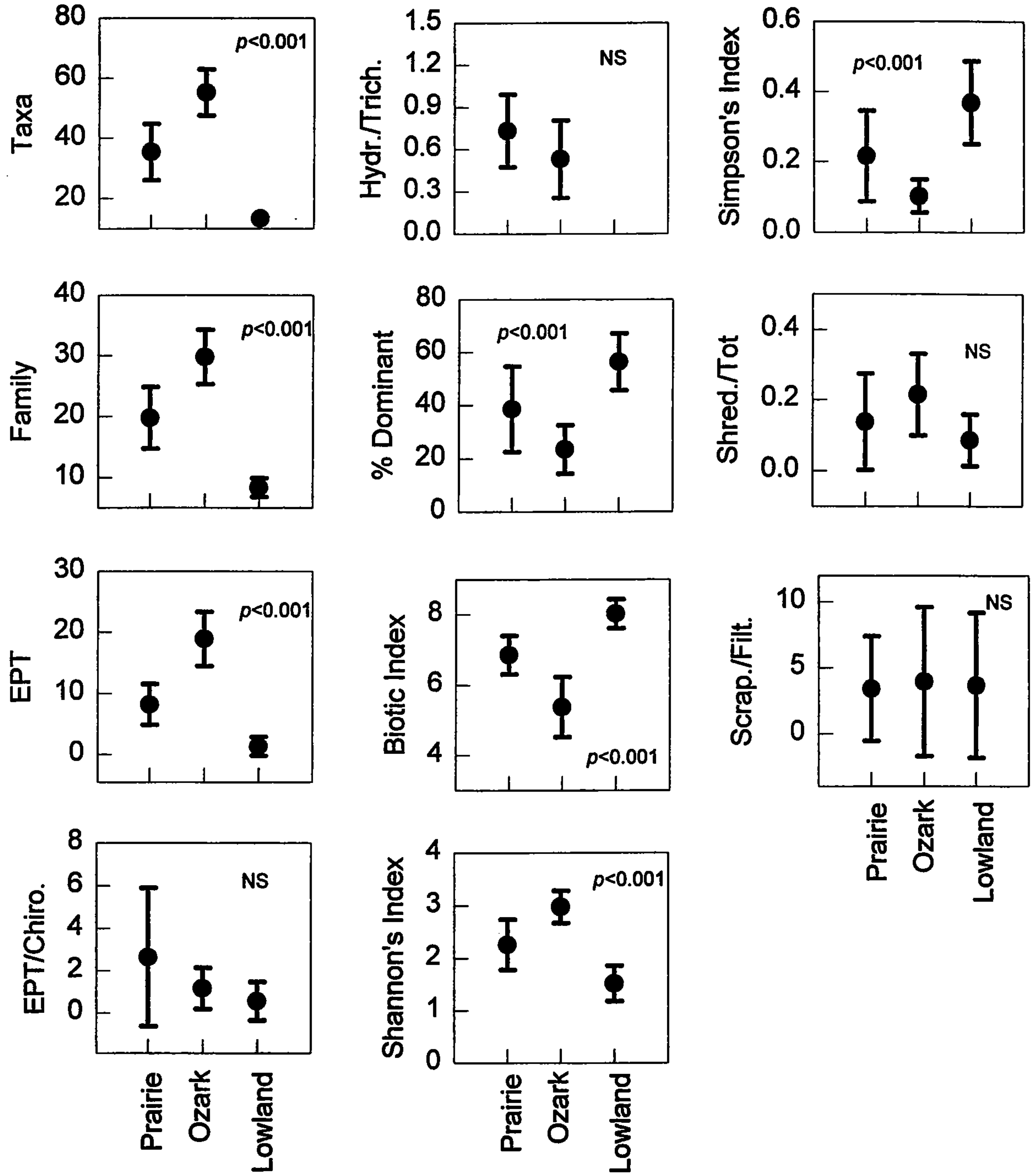


Fig. 3. A comparison of among region differences of common metrics from reference sites, spring 1993, all habitats (mean and standard deviation) . Significance levels are for overall differences as determined by ANOVA.

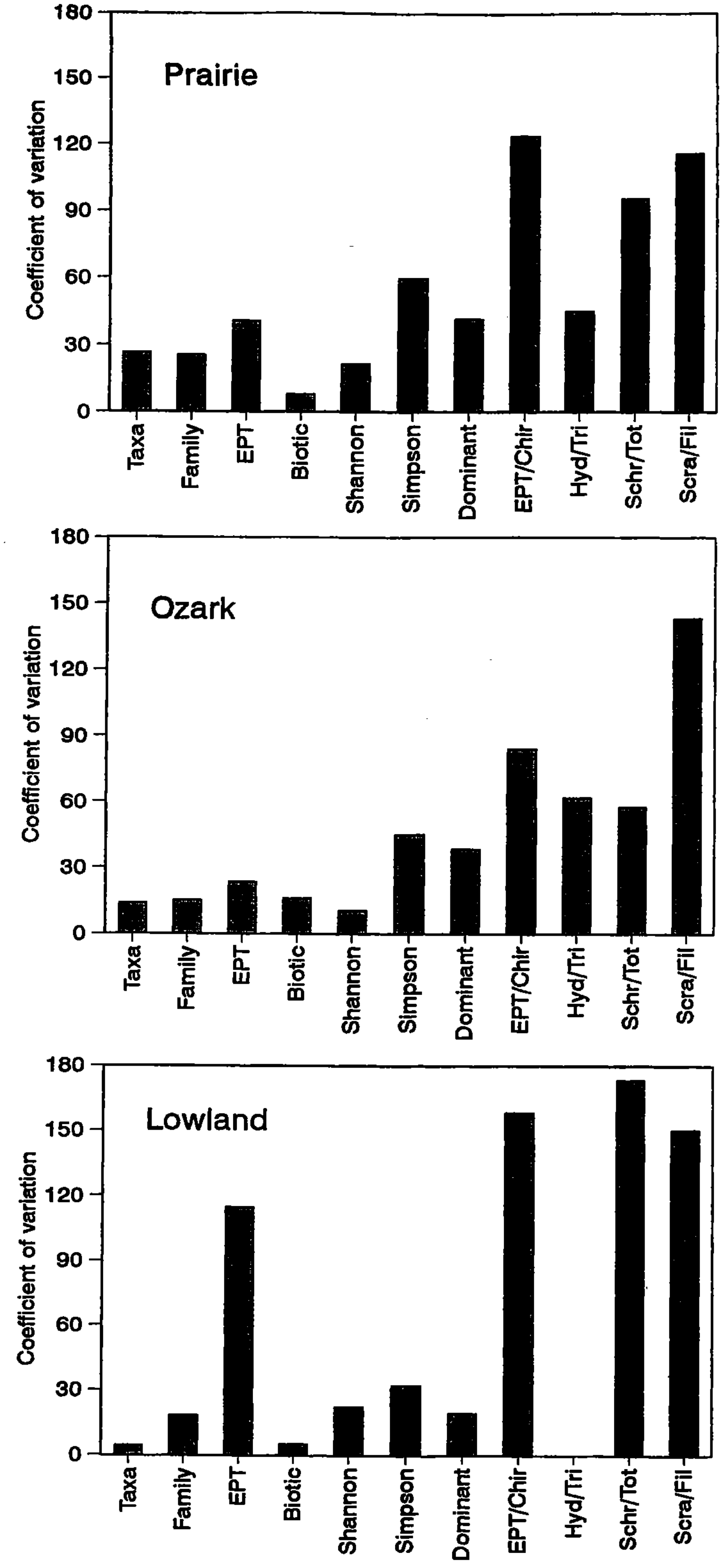


Fig. 4. Variations of 11 metrics from reference streams of each region, spring 1993.

taxonomic group to another or one functional group to another did not help us distinguish among geographical areas as well as the other seven metrics and would probably not be useful in developing a sensitive index. Further analyses were limited to: Total taxa, Family, EPT, BI, Shannon's Diversity index, Simpson's Diversity Index, and % Dominant taxon.

Effects of the Number of Different Habitats at a Site on Metric Values

Using all available habitat types at each site for biological criteria is often done. However, if the habitat types are different from site to site or among regions, the communities might be different just on that basis, rather than impairment. It seems appropriate to include all habitats if the objective is to characterize the actual community at a site. However, if the objective is to investigate effects of water quality it is obviously better to have a standardized number of habitats that the sites to be compared have in common.

Because we were interested in obtaining the best representation of the invertebrate community from each site, we sampled all available habitat types. But because not all habitats were present at every site there was the possibility of metrics being affected simply because of the number of available habitats. We evaluated how metric values related to the number of habitats at a site. In both the Ozark region (Fig. 5) and Prairie region (Fig. 6) results showed the influence of the number of habitats sampled on several metrics. Significant correlations were found only for the metrics total taxa and family ($P = 0.05$) but trends were evident for % Dominant taxon, BI, and Shannon's and Simpson's Diversity Indices, as well as many of the ratio metrics.

Because metric values probably were influenced by the number of habitats sampled, we confined ourselves for the remainder of the study in making comparisons to either using a single common habitat, or by using identical combinations of habitats, i.e., multihabitat sampling.

Community Structure—Multihabitat

Spring

Flowing water-coarse substrate (cs flow), nonflowing water (nonflow), and rootmats (root) were the most commonly sampled habitat types. We used those sites possessing the three common habitat types, 8 from the Prairie, and 17 from the Ozark, in this analysis. No streams from the lowland region was used because only one habitat was available per site. The DCA separated the two regions well (Fig. 7). No overlap was evident between the two regions.

Fall

All reference streams sampled in spring 1993 were sampled again in the fall, except Huffstetter Lateral Ditch, which was dry at the time. While all available habitats were sampled at each site (Table 2) only the streams that had three habitats (cs flow, nonflow, and rootmats) were used; i.e, 11 prairie and 25 Ozark were used in the multihabitat analysis. This is consistent with results of the multihabitat data analysis of spring.

Communities from the two regions were generally different as evidenced by the plots in Fig. 8a, but there was some overlap between communities from Prairie region streams and those from Ozark region streams. A clearer separation is noted if we designate several of the sites as transitional (Fig. 8b). These transitional streams are all either from the Ozark Border region as tributaries close to the Missouri River, or from the southeastern Ozarks.

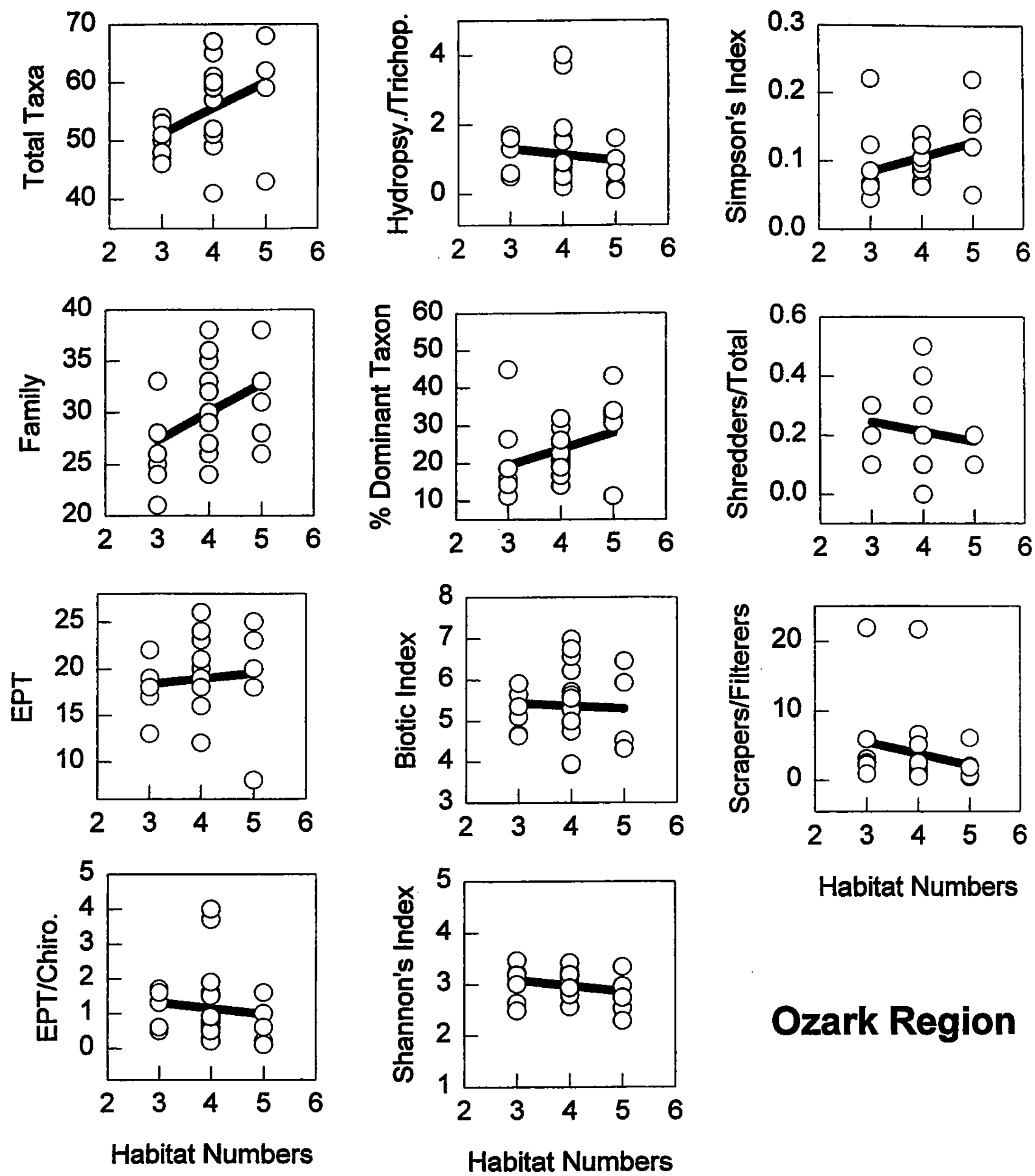


Fig. 5. The relation of metric values to the total number of habitats sampled in the Ozark reference streams, spring 1993.

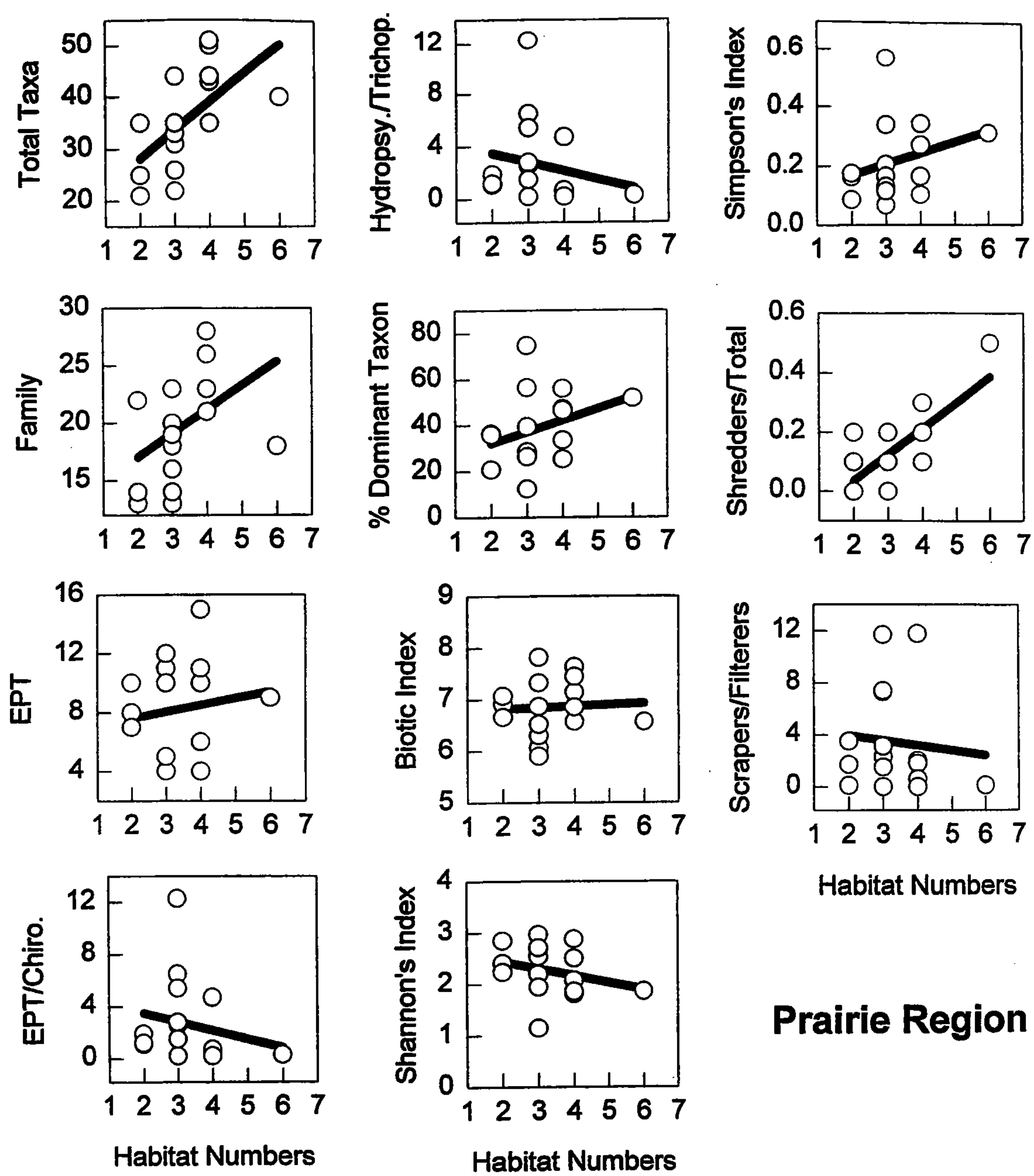


Fig. 6. The relation of metric values to the total number of habitats sampled in the Prairie reference streams, spring 1993.

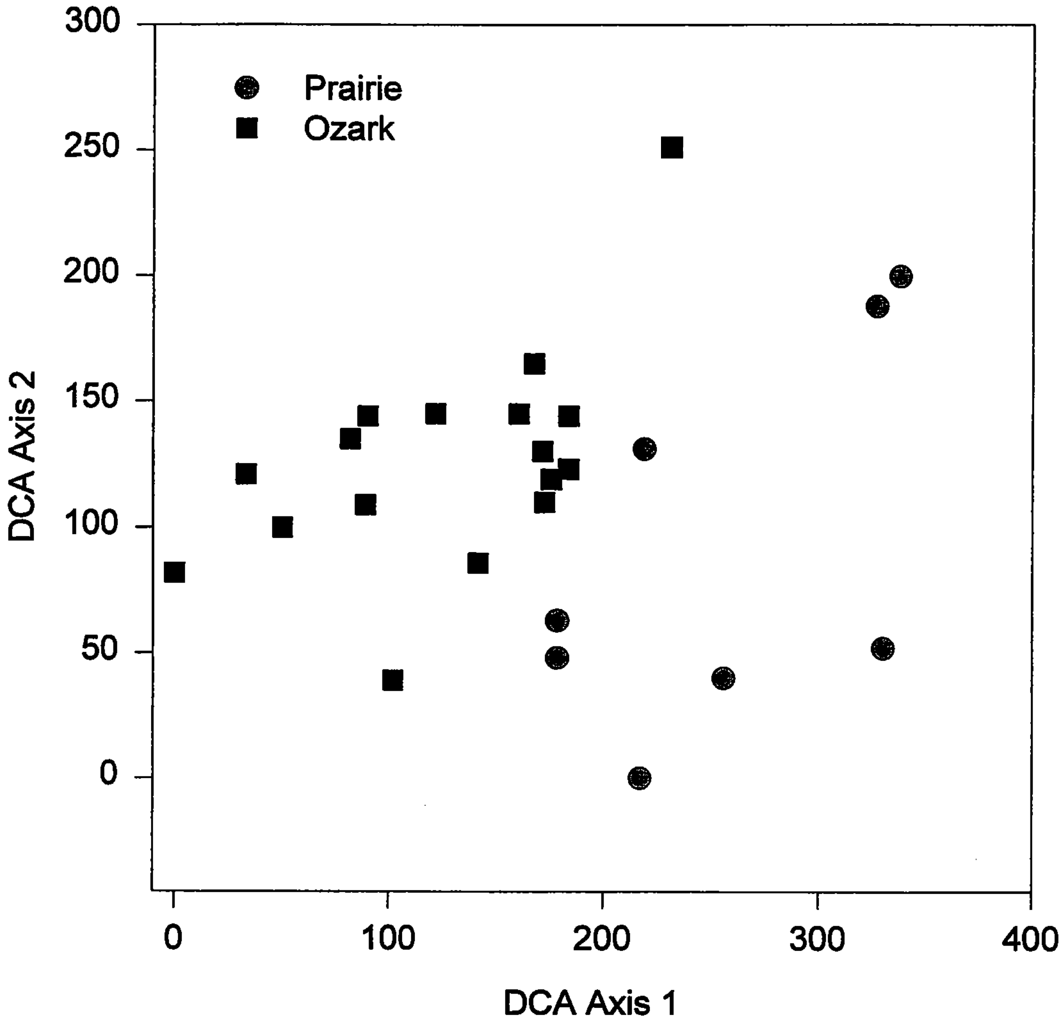


Fig. 7. Ordination of invertebrate communities from reference sites possessing habitats in common, spring 1993.

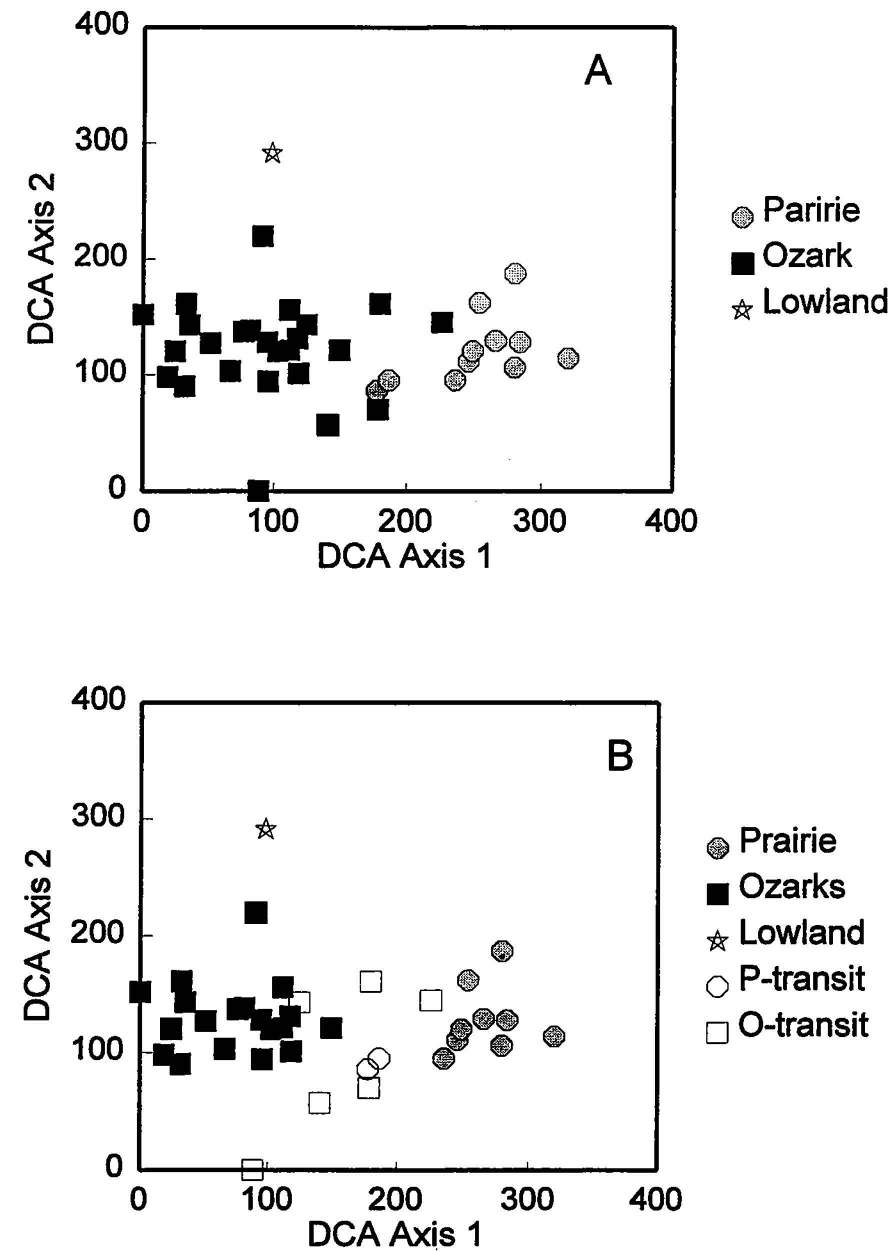


Fig. 8. Ordination of invertebrate communities from reference sites possessing habitats in common, fall 1993: A) all sites, B) geographical transitional sites indicated.

Comparison of Metrics Between Regions (multihabitat)

Spring

The seven metrics retained for further consideration were used to evaluate differences among regions using multihabitat data. All seven metrics showed significant differences between prairie and Ozark region streams (*t*-test, $P = 0.01$; Table 6). Additionally, the CV within a region for any particular metric was quite small (Table 6). All but two of the metrics had a CV less than 50%. The mean variation for the Prairie region was 27% and for the Ozark 19%.

Fall

Table 7 shows the value of seven metrics for each stream, their means, and CV by region. Similar to spring 1993, the means for all seven metrics were significantly different between Prairie and Ozark regions (*t*-test, $P < 0.05$) indicating all metrics were sensitive to regional differences. The CVs were highest for Simpson's diversity index, and % Dominant taxon but were low for the other five metrics An analysis identical to the above was done on a dataset where transitional streams were eliminated (Table 8). The results are quite similar to results when using all sites, and conclusions from using either dataset would be the same. However, variation of the metrics calculated without the transition streams was somewhat lower in most cases. This aspect of the study indicates that the seven metrics had quite low variation and were able to detect regional differences.

Correlation with Water Quality and Habitat Variables

Spring

Correlation analyses using all data from spring 1993 indicate strong associations between metric scores and environmental data (Table 9). Of the 11 metrics, 10 were significantly correlated with total nitrogen (TN), 10 with TP, and 8 with habitat score. A caveat is needed, however, because the prairie region was consistently high in nutrients and low in habitat score. So it is doubtful that the data is independent and that there are not a lot more cocorrelates involved that we did not measure.

When analyzed by ecoregion, some associations are still strong (Table 10). Of the 22 possible associations 3 were significant for nitrogen, 1 for habitat, but 7 for phosphorus. At this point in the analyses, just using reference streams, we would rather see no significant relations among these variables, but apparently reference conditions were variable enough that some possible effect of enrichment was noted.

To better show relations in Tables 9 and 10 among physical habitat and the metrics, graphical presentations were developed (Fig. 9). When data from both regions are used, most metrics show significant results. There are especially strong relations for Total taxa, Family, EPT, % Dominant taxon, Simpson's diversity index, and the BI. Thus our conclusion that higher habitat scores relates to "better" metric scores. However, in practically every case, regions grouped by themselves, with lowland having the worst habitat scores, prairie in between,

Table 6. Metrics for each reference stream in spring 1993. Analysis done by using multihabitat data, where every site had three habitats in common: cs flow, nonflow and root mat.

Streams	Str.No.	Region	Taxa	Family	EPT	Biotic Ind	Shannon	Simpson	Dominant
White	1	Prairie	31	18	4	7.3	2.22	0.21	39.5
Honey	3	Prairie	22	13	5	6.1	2.19	0.17	26.6
Marrowbone	7	Prairie	46	26	14	7.6	1.69	0.37	58.6
West Locust	9	Prairie	39	17	9	6.9	2.45	0.17	35.4
Spring	10	Prairie	32	19	9	7.1	1.93	0.29	50.1
North	13	Prairie	47	24	11	6.6	2.84	0.11	25.8
Petite Sal.	15	Prairie	32	21	5	7.0	2.04	0.21	37.3
Loutre	16	Prairie	40	25	3	7.6	2.49	0.17	36.4
Means			**36.1**	**20.4**	**7.5**	**7.0**	**2.23**	**0.21**	**38.7**
SD			**8.4**	**4.5**	**3.9**	**0.5**	**0.36**	**0.08**	**11.1**
C.V.			**23.4**	**21.9**	**51.4**	**7.5**	**16.2**	**39.9**	**28.6**
R.Aux Vasse	17	Ozark	61	31	19	6.3	3.20	0.09	25.7
Apple	18	Ozark	44	25	11	6.5	2.90	0.09	19.6
Saline	19	Ozark	53	27	19	6.3	2.64	0.15	33.6
Boeuf	22	Ozark	38	24	7	6.6	2.33	0.20	41.3
Cedar	23	Ozark	47	26	18	5.9	3.16	0.07	15.8
Pomme	24	Ozark	53	32	14	6.2	3.19	0.07	16.6
Deer	25	Ozark	53	33	16	5.6	3.16	0.07	16.7
Little Niang.	26	Ozark	57	30	16	6.7	3.06	0.09	19.4
Bull	29	Ozark	48	25	18	5.1	3.22	0.06	15.7
Spring(Doug.	30	Ozark	60	28	16	5.2	3.44	0.05	12.8
Sinking(Sha)	33	Ozark	47	23	14	4.4	3.26	0.05	12.0
Little black	35	Ozark	56	31	16	5.4	3.11	0.09	24.4
Little piney	36	Ozark	51	33	22	5.9	2.49	0.22	45.0
Meremac	38	Ozark	61	33	21	5.0	3.17	0.09	24.9
Huzzah	39	Ozark	47	27	22	3.8	2.70	0.12	21.0
Marble	40	Ozark	63	37	20	5.2	3.41	0.05	11.9
Sinking(Rey)	42	Ozark	48	29	16	4.2	2.63	0.17	37.0
Means			**52.2**	**29.1**	**16.8**	**5.6**	**3.00**	**0.10**	**23.1**
SD			**6.9**	**3.9**	**3.9**	**0.9**	**0.33**	**0.05**	**10.3**
C.V.			**13.2**	**13.4**	**23.2**	**15.6**	**10.9**	**51.7**	**44.6**
Difference between Prairie (n=8) and Ozark (n=17) Region.									
t-test, p values			**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.008**	**0.005**

Table 7. Metrics for each reference stream in fall 1993. Analyses done by multihabitat, where every site had three habitats in common: cs flow, nonflow and root mat.

Stream	No.	Region	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
White Cloud	1	Prairie	47	27	11	6.0	2.69	0.13	24.2
Honey	3	Prairie	42	19	15	5.8	2.30	0.18	36.2
Grindstone	5	Prairie	38	24	13	6.1	2.86	0.10	24.3
W.Fk.Big	6	Prairie	50	21	16	5.5	2.79	0.10	24.1
No Cr.	8	Prairie	38	20	9	6.5	2.05	0.26	47.3
W. Locust	9	Prairie	35	15	9	5.9	1.85	0.32	53.9
E. Fk. Crooked	11	Prairie	47	23	8	6.2	2.55	0.14	30.1
Mid. Fabius	12	Prairie	32	23	10	6.2	2.61	0.11	23.3
North R.	13	Prairie	40	19	11	6.5	2.50	0.13	22.5
Petite Saline	15	Prairie	32	18	9	6.8	2.69	0.10	17.3
Loutre	16	Prairie	33	16	7	6.7	2.62	0.11	21.3
MEANS			**39.5**	**20.5**	**10.7**	**6.2**	**2.50**	**0.15**	**29.5**
SD			**6.4**	**3.6**	**2.9**	**0.4**	**0.31**	**0.07**	**11.6**
C.V.			**16.2**	**17.5**	**26.7**	**6.4**	**12.5**	**47.7**	**39.3**
R. Aux Vasse	17	Ozarks	51	23	15	6.1	3.13	0.08	18.7
Apple	18	Ozarks	60	34	15	5.9	2.93	0.11	24.7
Saline	19	Ozarks	52	33	18	5.8	3.03	0.09	17.9
Burris	21	Ozarks	48	26	13	6.1	3.13	0.06	10.8
Bouef	22	Ozarks	42	21	15	6.0	2.91	0.10	24.3
Cedar	23	Ozarks	51	27	11	6.7	3.21	0.06	14.3
Pom. de Terre	24	Ozarks	53	34	16	6.6	2.95	0.10	23.7
Deer Cr.	25	Ozarks	54	34	14	5.8	3.45	0.04	8.8
Ltl. Niangua	26	Ozarks	45	29	15	5.6	3.25	0.05	10.5
Ltl. Maries	27	Ozarks	48	27	14	5.7	2.83	0.09	17.8
Big Sugar	28	Ozarks	52	31	22	4.6	3.07	0.09	22.0
Bull	29	Ozarks	49	25	14	4.5	2.90	0.10	22.1
Spring (Doug.)	30	Ozarks	56	27	14	4.3	3.00	0.10	25.1
North Fork	31	Ozarks	59	30	20	5.5	3.44	0.05	10.4
Jacks Fork	32	Ozarks	52	29	14	4.4	2.93	0.11	27.1
Sinking (Shan.	33	Ozarks	52	30	19	4.3	2.34	0.27	50.7
Big Cr.	34	Ozarks	56	26	16	4.6	3.34	0.05	11.0
Ltl. Black	35	Ozarks	62	31	13	5.5	3.31	0.06	13.6
W. Piney	36	Ozarks	53	29	15	5.2	2.80	0.10	19.0
Ltl. Piney	37	Ozarks	45	28	14	4.7	2.88	0.10	23.6
Meramec	38	Ozarks	65	33	19	5.6	3.31	0.05	11.7
Huzzah	39	Ozarks	57	29	18	5.4	3.26	0.07	21.7
Marble	40	Ozarks	68	32	21	6.0	3.32	0.06	17.7
E. Fk. Black	41	Ozarks	55	34	20	4.8	3.45	0.05	10.4
Sinking (Reyn.)	42	Ozarks	54	31	17	4.4	3.14	0.11	30.3
MEANS			**53.6**	**29.3**	**16.1**	**5.4**	**3.09**	**0.09**	**19.5**
SD			**6.1**	**3.5**	**2.8**	**0.7**	**0.26**	**0.04**	**9.0**
C.V.			**11.4**	**12.0**	**17.6**	**13.6**	**8.3**	**51.7**	**45.9**
Difference between Prairie (n=11) and Ozarks (n=25) region.									
t-test, P values			**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.014**	**0.022**
Maple Sl.	45	Map	38	22	8	6.1	2.60	0.16	35.3

Table 8. Metrics for each reference stream in fall 1993. Analyses done by multihabitat, where every site had three habitats in common: cs flow, nonflow and root mat. Analysis was conducted after elimilating transitional streams.

Stream	No.	Region	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
White Cloud	1	Prairie	47	27	11	6.0	2.69	0.13	24.2
Honey	3	Prairie	42	19	15	5.8	2.30	0.18	36.2
Grindstone	5	Prairie	38	24	13	6.1	2.86	0.10	24.3
W.Fk.Big	6	Prairie	50	21	16	5.5	2.79	0.10	24.1
No Cr.	8	Prairie	38	20	9	6.5	2.05	0.26	47.3
W. Locust	9	Prairie	35	15	9	5.9	1.85	0.32	53.9
E. Fk. Crooked	11	Prairie	47	23	8	6.2	2.55	0.14	30.1
Mid. Fabius	12	Prairie	32	23	10	6.2	2.61	0.11	23.3
North R.	13	Prairie	40	19	11	6.5	2.50	0.13	22.5
MEANS			**41.0**	**21.2**	**11.3**	**6.1**	**2.47**	**0.16**	**31.8**
SD			**6.0**	**3.5**	**2.8**	**0.3**	**0.34**	**0.08**	**11.6**
C.V.			**14.7**	**16.5**	**24.6**	**5.3**	**13.8**	**47.0**	**36.6**
Cedar	23	Ozarks	51	27	11	6.7	3.21	0.06	14.3
Pom. de Terre	24	Ozarks	53	34	16	6.6	2.95	0.10	23.7
Deer Cr.	25	Ozarks	54	34	14	5.8	3.45	0.04	8.8
Ltl. Niangua	26	Ozarks	45	29	15	5.6	3.25	0.05	10.5
Big Sugar	28	Ozarks	52	31	22	4.6	3.07	0.09	22.0
Bull	29	Ozarks	49	25	14	4.5	2.90	0.10	22.1
Spring (Doug.)	30	Ozarks	56	27	14	4.3	3.00	0.10	25.1
North Fork	31	Ozarks	59	30	20	5.5	3.44	0.05	10.4
Jacks Fork	32	Ozarks	52	29	14	4.4	2.93	0.11	27.1
Sinking (Shan.	33	Ozarks	52	30	19	4.3	2.34	0.27	50.7
Big Cr.	34	Ozarks	56	26	16	4.6	3.34	0.05	11.0
Ltl. Black	35	Ozarks	62	31	13	5.5	3.31	0.06	13.6
W. Piney	36	Ozarks	53	29	15	5.2	2.80	0.10	19.0
Ltl. Piney	37	Ozarks	45	28	14	4.7	2.88	0.10	23.6
Meramec	38	Ozarks	65	33	19	5.6	3.31	0.05	11.7
Huzzah	39	Ozarks	57	29	18	5.4	3.26	0.07	21.7
Marble	40	Ozarks	68	32	21	6.0	3.32	0.06	17.7
E. Fk. Black	41	Ozarks	55	34	20	4.8	3.45	0.05	10.4
Sinking (Reyn.)	42	Ozarks	54	31	17	4.4	3.14	0.11	30.3
MEANS			**54.6**	**29.9**	**16.4**	**5.2**	**3.12**	**0.09**	**19.7**
SD			**5.9**	**2.7**	**3.1**	**0.8**	**0.28**	**0.05**	**10.0**
C.V.			**10.8**	**9.0**	**18.6**	**14.5**	**9.0**	**59.1**	**50.7**
Difference between Prairie (n=9) and Ozarks (n=19) region.									
t-test, P values			**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.017**	**0.018**
Maple Sl.	45	Map	38	22	8	6.1	2.60	0.16	35.3

Table 9. Correlation coefficients and their associated probabilities (p) between metrics and total nitrogen, total phosphorus and habitat score, spring 1993: all sites from three ecoregions combined.

	Tot. Nitr.	Tot. Phos.	Hab. Score
TAXA	-0.413	-0.852	0.807
p	0.005	0.000	0.000
N	44	44	44
FAMILY	-0.395	-0.791	0.802
	0.008	0.000	0.000
	44	44	44
EPT	-0.469	-0.838	0.795
	0.001	0.000	0.000
	44	44	44
EPT/CHIR	0.461	0.420	-0.124
	0.002	0.005	0.421
	44	44	44
HYDR/TRI	0.486	0.452	-0.254
	0.002	0.004	0.125
	38	38	38
DOMINANT	0.319	0.539	-0.651
	0.035	0.000	0.000
	44	44	44
BIOTIC IND.	0.457	0.764	-0.803
	0.002	0.000	0.000
	44	44	44
SHANNON	-0.420	-0.701	0.753
	0.005	0.000	0.000
	44	44	44
SIMPSON	0.353	0.573	-0.644
	0.019	0.000	0.000
	44	44	44
SHRED/TOT	-0.302	-0.399	0.303
	0.046	0.007	0.045
	44	44	44
SCRAP/FIL	0.178	0.038	0.118
	0.247	0.806	0.444
	44	44	44

Table 10. Correlation coefficients and their associated probabilities (p) between metrics and total nitrogen (TN), total phosphorus (TP) and habitat score, spring 1993, by ecoregion.

	Prairie			Ozark		
	TN	TP	SCORE	TN	TP	SCORE
TAXA	-0.327	-0.706	0.013	-0.374	-0.377	0.257
p	0.216	0.002	0.962	0.066	0.063	0.216
N	16	16	16	25	25	25
FAMILY	-0.224	-0.559	0.167	-0.400	-0.241	0.234
	0.404	0.024	0.536	0.048	0.246	0.260
	16	16	16	25	25	25
EPT	-0.643	-0.686	-0.323	-0.161	-0.380	0.317
	0.007	0.003	0.222	0.441	0.061	0.123
	16	16	16	25	25	25
EPT/CHIR	0.439	0.680	0.075	0.172	-0.223	0.125
	0.089	0.004	0.783	0.411	0.285	0.553
	16	16	16	25	25	25
HYDR/TRI	0.556	0.709	0.275	0.237	-0.088	0.099
	0.031	0.003	0.322	0.277	0.688	0.654
	16	16	16	23	23	23
DOMINANT	0.204	0.025	-0.238	0.123	0.138	-0.172
	0.448	0.927	0.375	0.560	0.511	0.412
	16	16	16	25	25	25
BIOTIC IND	0.434	0.244	-0.211	0.344	0.545	-0.494
	0.093	0.363	0.433	0.092	0.005	0.012
	16	16	16	25	25	25
SHANNON	-0.295	-0.167	0.119	-0.349	-0.311	0.267
	0.267	0.537	0.661	0.088	0.130	0.197
	16	16	16	25	25	25
SIMPSON	0.261	0.113	-0.111	0.195	0.178	-0.214
	0.329	0.678	0.682	0.350	0.395	0.305
	16	16	16	25	25	25
SHRED/TOT	-0.468	-0.570	0.166	0.043	0.205	-0.280
	0.068	0.021	0.540	0.839	0.327	0.176
	16	16	16	25	25	25
SCRAP/FIL	0.163	0.354	0.456	0.385	-0.047	0.006
	0.545	0.179	0.076	0.057	0.822	0.977
	16	16	16	25	25	25

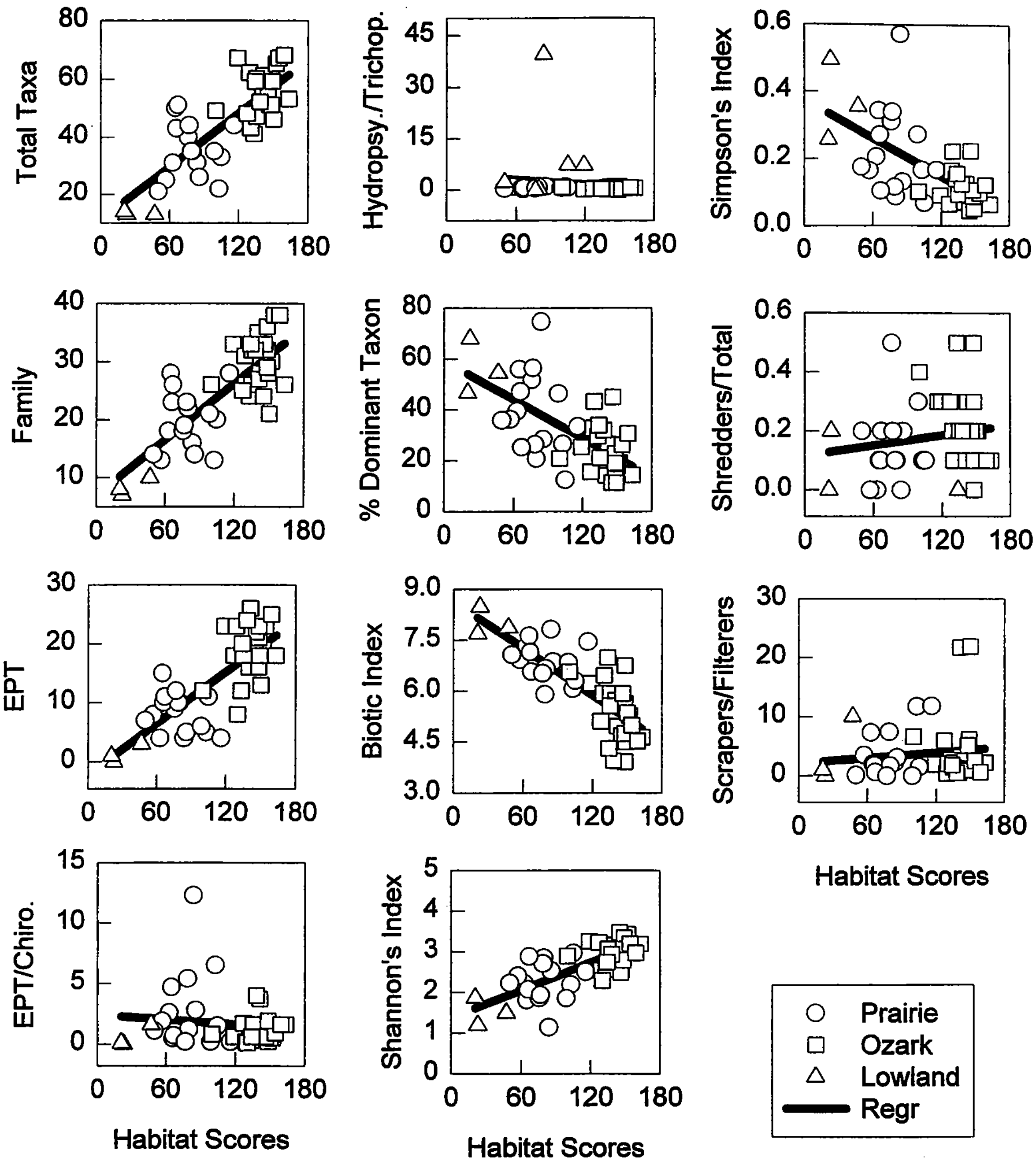


Fig. 9. The relation of habitat scores to individual metrics for all reference streams, spring 1993.

Table 11. Correlation coefficients and their associated probabilities (p) between metrics and totoal nitrogen (TN), total phosphorus (TP) and habitat score, multihabitat, fall 1993, two ecoregions combined and by ecoregion.

	All streams (N=37)			Prairie (N=11)			Ozark (N=25)		
	TN	TP	SCORE	TN	TP	SCORE	TN	TP	SCORE
TAXA	-0.363	-0.655	0.464	-0.305	-0.103	-0.487	-0.241	-0.275	0.044
p	0.027	0.000	0.004	0.362	0.764	0.129	0.246	0.183	0.836
FAMILY	-0.254	-0.557	0.496	0.041	0.188	-0.373	-0.070	-0.093	0.106
p	0.129	0.000	0.002	0.906	0.579	0.258	0.740	0.658	0.614
EPT	-0.175	-0.594	0.502	-0.151	0.303	-0.319	0.041	-0.342	0.157
p	0.301	0.000	0.002	0.658	0.365	0.339	0.846	0.095	0.454
BIOTIC IND	0.238	0.576	-0.553	0.478	0.037	0.277	0.055	0.524	-0.567
p	0.157	0.000	0.000	0.137	0.914	0.409	0.793	0.007	0.003
SHANNON	-0.314	-0.573	0.495	0.098	0.070	-0.046	-0.240	-0.209	-0.019
p	0.058	0.000	0.002	0.776	0.838	0.894	0.249	0.315	0.928
SIMPSON	0.146	0.383	-0.360	-0.065	0.002	-0.028	0.023	-0.064	0.075
p	0.390	0.019	0.029	0.851	0.995	0.935	0.913	0.762	0.722
DOMINANT	0.091	0.337	-0.319	-0.106	0.019	0.004	-0.006	-0.156	0.081
p	0.592	0.041	0.054	0.756	0.955	0.990	0.979	0.456	0.699

and Ozark the highest score. Within a region there were no significant relations between metrics and habitat score. This is as it should be for ecoregion-based reference conditions.

Fall

Correlation analyses using data combined from all regions from the fall period indicated many significant associations (Table 11). Of the seven metrics, all were significantly or marginally correlated with habitat score and TP, while one was significantly correlated with TN. However we must caution that some of these relations may be spurious because nutrient concentrations and habitat scores were strongly related to region, so there is very good reason to believe that numerous cocorrelates were not measured. An analysis by region (Table 11) indicates many fewer significant associations. Only two relations from the Ozark region, one with BI and TP and the other BI and habitat score, were significant.

To better show relations in Table 11 among physical habitat and some of the metrics, graphical presentations were developed (Fig. 10). When all sites are used from all regions, most metrics show significant relations. Especially strong relations are seen with the metrics: Total taxa, Family, EPT, BI, and Shannon's diversity index. Higher habitat scores were related to "better" scores for the metrics. Again the results are primarily due to geographical groupings—generally lower habitat scores for prairie and higher for Ozark. Strong within-region associations are not evident.

CONCLUSIONS

Benthic invertebrates collected from reference streams have a typical regional fauna, which relates well to the main ecoregions of the state: Ozark, Prairie, and the Lowland area (Mississippi Alluvial Plain). Subregionalization is probably not necessary. In fact, similarity analyses (data not presented) indicated little improvement in reducing variation from the three main ecoregions. Within each region reference stream, communities are similar and possess relatively low variation, probably due to care in site selection, timing of sampling, and strictly adhering to sampling protocols. Metrics found most useful to describe invertebrate communities were Total taxa, Family, EPT, BI, Shannon's diversity index, Simpson's diversity index, and % Dominant taxon. These metrics were statistically different among regions and had remarkably low variation. These metrics were chosen as candidates for further analysis.

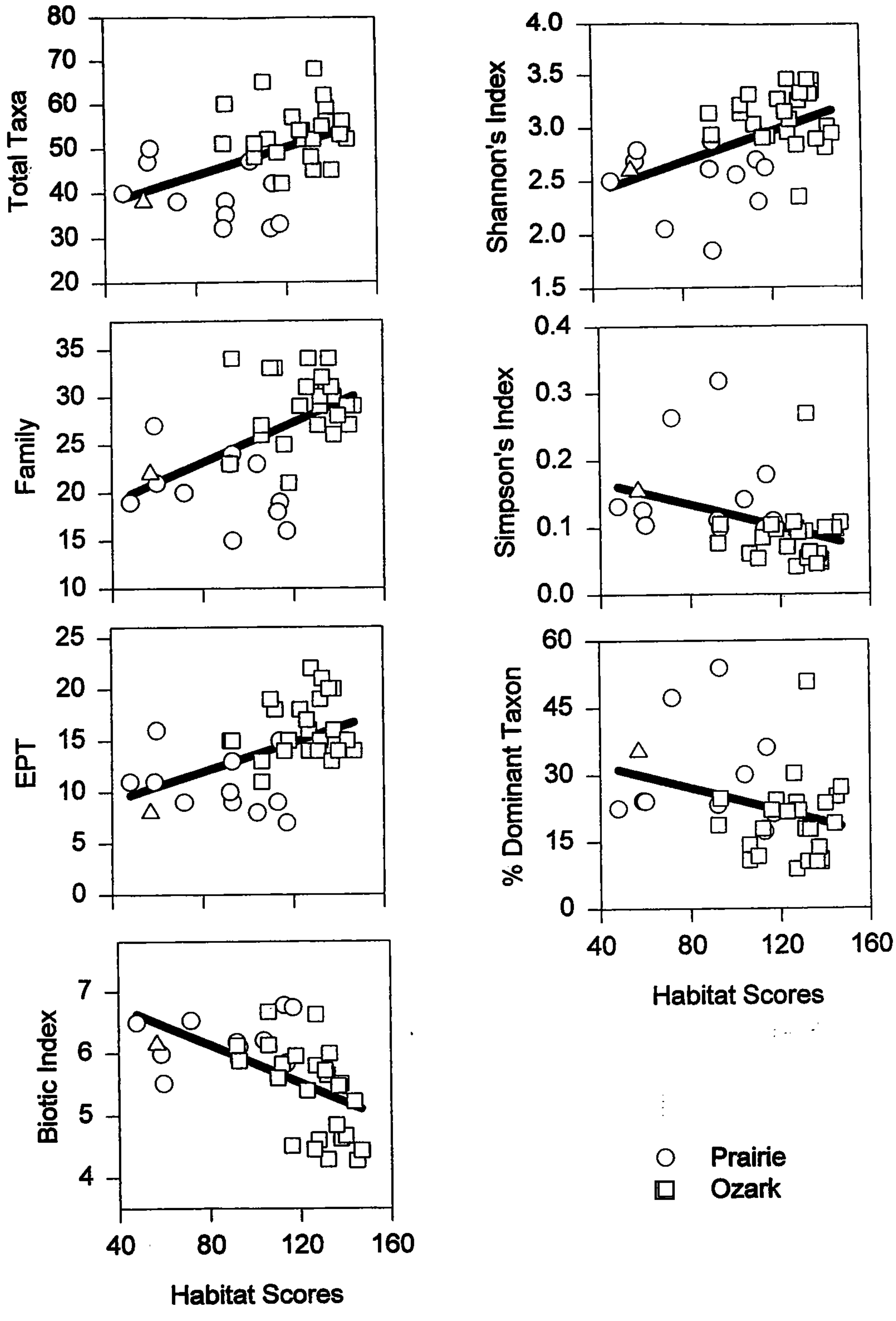


Fig. 10. The relation of habitat scores to individual metrics for all reference streams, fall 1993.

Chapter 8

EVALUATING METRIC SENSITIVITY

INTRODUCTION

Metrics selected for use in a biocriteria program either singly or combined for inclusion in a Stream Condition Index (SCI) must possess low variability and high sensitivity. Variability of metrics was previously examined using the reference site collections of 1993 where from an initial suite of 11 metrics, 7 were retained as candidates to be used either singly or combined in a final index. Metric sensitivity, which is the ability to discriminate between reference and impaired sites is addressed in this chapter. Part A of this chapter uses the fall 1994 dataset, while Part B uses the 1995 dataset. Part C of this chapter evaluates which metrics are redundant.

Previous activities of this project emphasized development and evaluation of reference conditions. Regionalization, methods development, methods evaluation, and metric characteristics have all been addressed. Now we intend to conduct sensitivity analyses by comparing reference conditions to situations we deem impaired. Because biological integrity relates to more than water quality conditions, we were interested in evaluating overall impairment (Karr 1981). While we believe biological integrity involves water quality, physical habitat conditions, flow regimes, biotic interactions, and appropriate balances of energy sources and flows, we decided to evaluate two most readily measurable characteristics: water quality, primarily organic enrichment as measured by dissolved nutrients; and physical habitat degradation.

We were not interested in providing a system that works under the worst conditions—most any system would. Certainly highly septic situations that kill off a majority of the benthos are readily apparent and need no further examination. We were more interested in examining conditions of what might be termed moderately affected—where problems are not immediately obvious to eye or nose.

PART A. EVALUATING METRIC SENSITIVITY TO IMPAIRMENT BY ORGANIC ENRICHMENT AND BY PHYSICAL HABITAT DEGRADATION—FALL 1994

In both the Prairie and Ozark regions, five reference streams (REF), five organically enriched streams (ORG), and five habitat degraded streams (HAB) were selected for study (Fig. 1, Table 1). For each stream, two sites adjacent to one another were sampled to decrease possible variation and make it easier to distinguish between REF and impaired (IMP) conditions (see Chapter 9). Data from replicate sites were examined separately to evaluate community structure but were averaged to calculate metrics prior to final analysis. All available habitats were sampled at each site (Table 2). Water quality samples were taken and habitat scores determined (Table 3). Sites were selected and categorized *a priori* using our best professional judgement.

Results were first examined to determine relative similarity of sites using DCA ordination. This was followed by sensitivity analysis of the metrics by: 1) examining the difference of impacted streams as a percent difference from a mean REF condition, 2) a statistical test for differences in mean values between REF

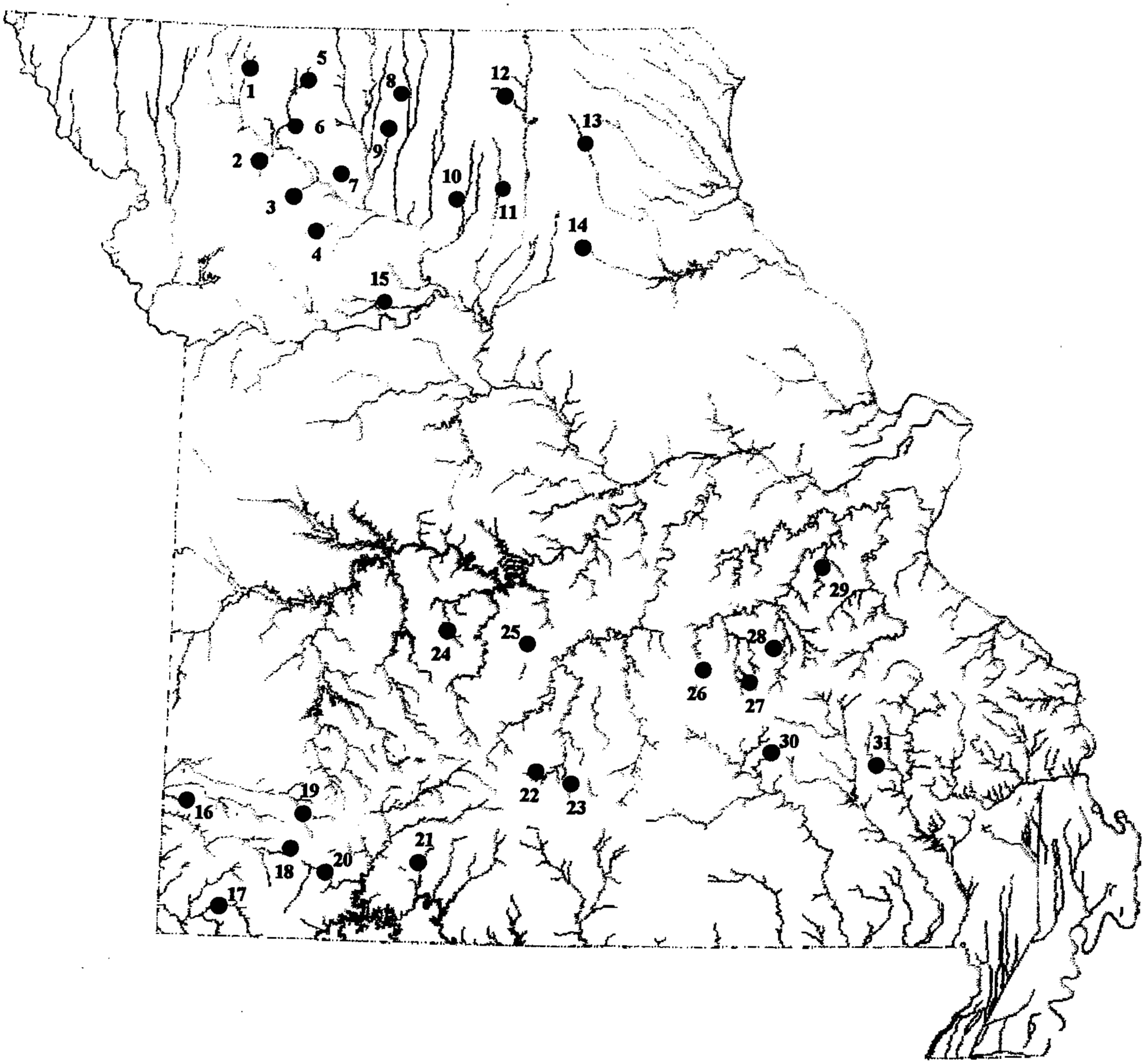


Fig. 1. Sampling sites for the fall 1994 survey.

Table 1. Fall 1994 sampling locations

Stream #	Designated stream condition	Stream	Site location	Comments
1	Reference	East Fk. Grand R.	Worth Co.; Sec. 32; T66N; R30W	
		East Fk. Grand R.	Worth Co.; border Secs. 12 & 13; T65N; R31W	
2	Reference	Grindstone Cr.	DeKalb Co.; border Secs. 2 & 3; T58N; R30W	
		Grindstone Cr.	DeKalb Co.; Sec. 24; T59N; R30W	
3	Reference	Marrowbone Cr.	Daviess Co.; border Secs. 5 & 8; T58N; R27W	
		Marrowbone Cr.	Daviess Co.; E 1/2; Sec. 7; T58N; R27W	1/2 mi. upstream of fallen bridge
4	Nutrient enrichment	Shoal Cr.	Caldwell Co.; Sec. 9; T56N; R26W	
		Shoal Cr.	Caldwell Co.; border Secs. 12 & 13; T56N; R26W	
5	Nutrient enrichment	East Fk. Big Cr.	Harrison Co.; N 1/2; Sec. 4; T65N; R27W	
		East Fk. Big Cr.	Harrison Co.; Sec. 24; T66N; R27W	
6	Habitat disturbance	Big Cr.	Daviess Co.; Sec. 1; T61N; R29W	
		Big Cr.	Daviess Co.; Sec. 23; T61N; R29W	
7	Nutrient enrichment	Big Muddy Cr.	Daviess Co.; border Secs. 11 & 14; T59N; R27W	
		Big Muddy Cr.	Daviess Co.; Sec. 3; T59N; R27W	
8	Habitat disturbance	West Fk. Medicine R.	Grundy Co.; S 1/2; Sec. 6; T62N; R22W	
		West Fk. Medicine R.	Grundy Co.; N border; Sec. 4; T62N; R22W	
9	Reference	No Cr.	Livingston Co.; T59N; border R23W & R24W	
		No Cr.	Grundy Co.; border Secs. 20 & 29; T60N; R23W	
10	Nutrient enrichment	West Yellow R.	Linn Co.; border Secs. 9 & 16: T57N; R19W	
		West Yellow R.	Linn Co.; border Secs. 21 & 28; T57N; R19W	
11	Habitat disturbance	Mussel Fk.	Chariton Co.; border Secs. 13 & 24; T56N; R18W	
		Mussel Fk.	Linn Co.; border Secs. 25 &36; T57N; R18W	
12	Reference	Spring Cr.	Adair Co.; Sec. 24; T63N; R17W	
		Spring Cr.	Adair Co.; Sec. 19; T63N; R17W	
13	Habitat disturbance	North Fk. Salt R.	Adair Co.; border Secs. 9 & 16; T61N; R13W	
		North Fk. Salt R.	Adair Co.; border Secs. 31 & 32; T62N; R13W	
14	Nutrient enrichment	Middle Fk. Salt R.	Macon Co.; border Secs. 9 & 16; T56N; R13W	
		Middle Fk. Salt R.	Macon Co.; Sec. 16; T56N; R13W	
15	Habitat disturbance	Wakenda Cr.	Carroll Co.; Sec. 10; T52N; R23W	
		Wakenda Cr.	Carroll Co.; Sec. 13; T52N; R23W	
16	Nutrient enrichment	Turkey Cr.	Jasper Co.; border Secs. 28 & 29; T28N; R33W	at the Joplin STP
		Turkey Cr.	Jasper Co.; border Secs. 29 & 30; T28N; R33W	
17	Reference	Big Sugar Cr.	McDonald Co.; Sec. 22; T22N; R30W	
		Big Sugar Cr.	McDonald Co.; border Secs. 1 & 12; T21N; R30W	
18	Nutrient enrichment	Clear Cr.	Barry Co.; Sec. 35; T26N; R28W	at the Monnett STP
		Clear Cr.	Barry Co.; Sec. 26; T26N; R28W	
19	Habitat disturbance	Spring R.	Lawrence Co.; N 1/2; Sec. 11; T27N; R27W	
		Spring R..	Lawrence Co.; E 1/2; Sec. 25; T27N; R27W	
20	Habitat disturbance	Flat Cr.	Barry Co.; E 1/2; Sec. 15; T24N; R26W	
		Flat Cr.	Barry Co.; Sec. 6; T24N; R26W	
21	Reference	Bull Cr.	Christian Co.; Sec. 36; T25N; R21W	
		Bull Cr.	Christian Co.; NW 1/4; Sec. 2; T24N; R21W	
22	Habitat disturbance	Woods Fk.	Wright Co.; Sec. 3; T29N; R15W	upstream of bridge
		Woods Fk.	Wright Co.; Sec. 3; T29N; R15W	downstream of bridge
23	Nutrient enrichment	Whetstone Cr.	Wright Co.; Sec. 8; T29N; R13W	
		Whetstone Cr.	Wright Co.; Sec. 21; T29N; R13W	
24	Reference	Little Niangua R.	Hickory Co.; N 1/2; Sec. 2; T37N; R20W	
		Little Niangua R.	Camden Co.; S 1/2; Sec. 19; T37N; R19W	
25	Nutrient enrichment	Dry Auglaize Cr.	Laclede Co.; Sec 30; T35N; R15W	
		Dry Auglaize Cr.	Laclede Co.; NE 1/4; Sec. 31; T35N; R15W	
26	Nutrient enrichment	Spring Br.	Dent Co.; border Secs. 2 & 3; T43N; R6W	
		Spring Br.	Dent Co.; Sec 32; T35N; R6W	
27	Habitat disturbance	Hutchins Cr.	Dent Co.; NW 1/4; Sec. 10; T34N; R4W	downstream of bridge
		Hutchins Cr.	Dent Co.; SE 1/4; Sec. 10; T34N; R4W	upstream of bridge
28	Reference	Huzzah Cr.	Crawford Co.; NW 1/4; Sec. 20; T36; R2W	
		Huzzah Cr.	Crawford Co.; SE 1/4; Sec. 20; T36N; R2W	
29	Habitat disturbance	Indian Cr.	Franklin Co.; Sec. 6; T41N; R1E	
		Indian Cr.	Franklin Co.; border Secs. 20 & 29; T41N; R1E	
30	Reference	Big Cr.	Shannon Co.; Sec. 7; T30N; R3W	
		Big Cr.	Shannon Co.; Sec. 32; T30N; R3W	
31	Nutrient enrichment	Big Cr.	Iron Co.; Sec. 22; T31N; R3W	
		Big Cr.	Iron Co.; Sec. 24; T31N; R3W	

Table 2. Habitats sampled at reference, habitat degraded and organically enriched Prairie streams. For each stream two sites adjacent to one another were sampled: X-site 1; O-site 2.

Stream	No.	cs flow	nonflow	root mat	snag	fs flow	vegetation
Reference streams							
E.Fk.Grand R.	1	X / O	X / O	X /	X / O	X / O	
Grindstone Cr.	2	X /	X / O	/ O	/ O	/ O	
Marrowb Cr.	3	X / O	X / O			X / O	
No Cr.	9		X / O	X /	X /	X / O	
Spring Cr.	12		X / O			X / O	/ O
Habitat degraded streams							
Big Cr.	6	/ O	X / O	X /	X /	X /	
W. Fk. Med. R.	8		X / O			X / O	
Mussel Fk.	11		X / O	X / O	X / O	X / O	
N.Fk.Salt R.	13		X / O	X /	X /	X / O	
Wakenda Cr.	15		X / O		X / O	X / O	
Organically enriched streams							
Shoal Cr.	4	/ O	X / O	X / O	X / O	X / O	
E.Fk.Big Cr.	5	/ O	X / O	X / O		X / O	
Big Muddy	7		X / O	X / O		X / O	
W. Yellow R.	10		X / O		X / O	X / O	
Mid.Fk.Salt R.	14	/ O	X / O	X / O	X /	X / O	

Table 3. Total nitrogen (TN, mg/L) and phosphorus (TP, ug/L) and habitat scores for Prairie streams, fall 1994

Stream	Site No.	TN			TP			Score		
		Site 1	Site 2	Mean	Site 1	Site 2	Mean	Site 1	Site 2	Mean
Reference streams										
E.Fk.Grand R.	1	0.62	0.42	0.52	78	91	85	117	91	104
Grindstone Cr.	2	0.50	0.45	0.48	112	114	113	95	101	98
Marrowb Cr.	3	0.48	0.27	0.38	56	44	50	92	125	109
No Cr.	9	0.82	0.75	0.79	128	144	136	114	97	106
Spring Cr.	12	0.56	0.26	0.41	46	10	28	108	122	115
Habitat degraded streams										
Big Cr.	6	0.46	0.88	0.67	80	79	80	111	98	105
Mussel Fk.	11	0.39	0.38	0.39	58	64	61	109	110	110
N.Fk.Salt R.	13	0.48	0.34	0.41	38	53	46	84	65	75
Wakenda Cr.	15	2.41	1.20	1.81	344	82	213	49	87	68
Organically enriched streams										
Shoal Cr.	4	0.70	1.14	0.92	87	124	106	118	104	111
E.Fk.Big Cr.	5	1.93	1.97	1.95	248	252	250	117	105	111
Big Muddy	7	0.45	0.71	0.58	138	144	141	67	79	73
W. Yellow R.	10	2.51	1.16	1.84	342	166	254	87	94	91
Mid.Fk.Salt R.	14	1.15	1.06	1.11	118	86	102	124	119	122

and IMP streams, and 3) an evaluation by the box and whisker plot method. The three steps of the sensitivity analysis were carried out for multihabitat and single habitat datasets from both Prairie and Ozark ecoregions. A HAB site, West Fork Med, was not used for data analysis because of an inadvertent mistake in processing samples.

Prairie Streams

Multihabitat (nonflow and fs flow)

Two prairie stream habitat types, nonflow and fs flow, could be found at most sites, other habitats were occasionally found at other sites (Table 2). For comparability, we used only data from the two common habitat types.

Community Structure

We first analyzed invertebrate community structure by DCA ordination. There was not total separation between REF sites and those considered to have habitat degradation (Fig. 2). This indicates that overall community structure of the two types of streams had many similarities. The group of ORG sites was somewhat, but not completely, separated from REF sites (Fig. 2). REF streams themselves were quite dispersed, indicating considerable variation in community structure.

Metric Sensitivity

Seven metrics were calculated for each site (Table 4), each value representing the mean of replicate sites of each stream except Grindstone Creek and Big Creek where one of the replicate sites did not have fs flow samples (Table 2), so for these streams only one site was used. To compare the metric value of each degraded stream to a reference condition we used mean metric values obtained from five REF streams as the reference condition. We assumed that the CVs represented the natural variation outside of which would be considered impaired (Table 4) and we rounded down to establish impact thresholds.

Values of IMP streams exceeded impairment thresholds in only a few cases. The BI and Total taxa did not indicate impairment in any instance. Other metrics indicated impairment only 22-44% of the time. This poses the question of whether the metrics were not sensitive or whether streams were not really impacted. The streams of this study had no long-term physical or chemical water quality data, so our judgement of enrichment was based on one or two water samples and "professional opinion." Table 3 shows nutrient and habitat scores for each site. Habitat scores of Big Creek and Mussel Fork sites were not lower than those of the REF stream, and water quality at the first Shoal Creek site was the same as the REFs. Results of the similarity comparison were relatively consistent with results of water quality and ordination: those streams differing from references shown by ordination were identified as impacted by the similarity analysis.

Statistical Analysis

We examined for differences in the mean values of each metric (Table 5). Significant differences between REF and HAB streams were found only for Family and EPT metrics (t-test $P < 0.05$). No significant differences were found for any of the metrics between REF and ORG sites ($P < 0.05$).

Box and Whisker Plots

Metric sensitivity was evaluated according to the degree of interquartile

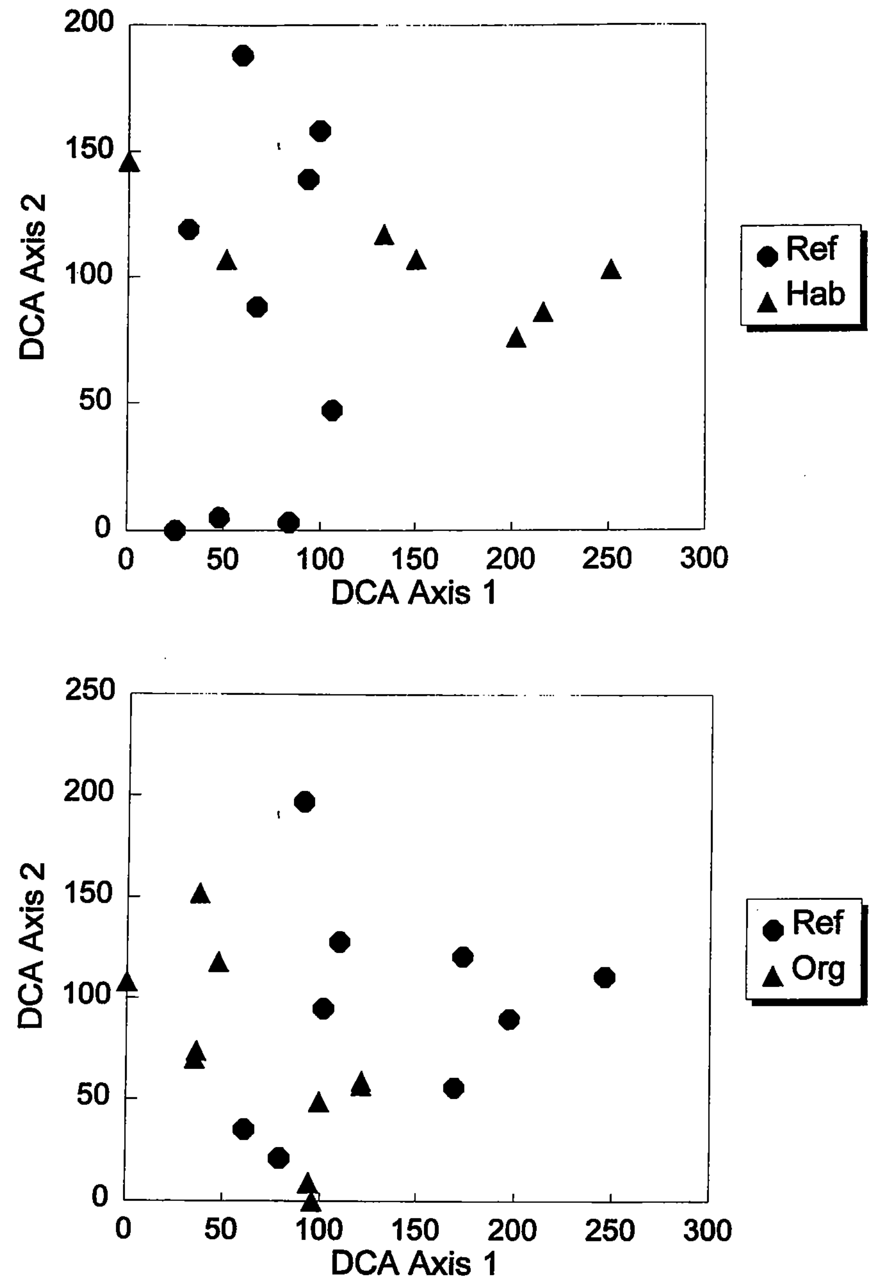


Fig. 2. Ordination of the reference (REF) and habitat degraded (HAB) sites, and the reference and organically enriched (ORG) sites, from the Prairie ecoregion, using multihabitat data, fall 1994.

Table 4. Metric values for three classes of streams and metic means and C.V. of 5 reference streams for multihabitat samples from Prairie streams, fall 1994. Impairment thresholds are based on the C.V. values. Percentage metric changes (italic numbers) of a degraded stream to mean values of reference streams exceeding thresholds are marked with stars.

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
E.Fk.Grand R.	ref	34.5	17.0	13.5	5.7	2.82	0.10	15.6
Grindstone Cr	ref	35.0	18.0	13.0	6.4	2.51	0.14	24.3
Marrowb Cr.	ref	35.5	17.0	9.5	6.1	2.63	0.14	30.3
No Cr.	ref	44.0	22.0	8.5	5.9	2.50	0.17	36.2
Spring Cr.	ref	37.5	18.0	10.0	6.1	2.67	0.12	24.1
MEAN		37.3	18.4	10.9	6.0	2.62	0.13	26.1
C.V.		10.5	11.3	20.4	4.1	5.00	19.37	29.5
Impact Threshold		85%	85%	75%	95%	95%	80%	70%
Big Cr.	hab	35.0 *93.8*	14.0 *76.1* *	9.0 *82.6*	6.3 *96.1*	2.15 *82.1* *	0.28 *48.2* *	16.8 *155.4*
Mussel Fk.	hab	36.0 *96.5*	16.0 *87.0*	8.5 *78.0*	6.2 *96.6*	2.63 *100.2*	0.12 *115.2*	22.8 *114.5*
N.Fk.Salt R.	hab	36.0 *96.5*	17.0 *92.4*	6.5 *59.6*	5.8 *104.2*	2.54 *96.7*	0.15 *86.9*	31.6 *82.6*
Wakenda Cr.	hab	33.5 *89.8*	15.0 *81.5* *	5.0 *45.9* *	6.3 *95.5*	2.26 *85.9* *	0.16 *85.8*	26.8 *97.4*
Shoal Cr.	org	34.5 *92.5*	17.0 *92.4*	12.0 *110.1*	6.2 *97.9*	2.75 *104.6*	0.11 *122.6*	26.0 *100.6*
E.Fk.Big Cr.	org	39.5 *105.9*	18.0 *97.8*	8.5 *78.0*	6.0 *100.6*	2.22 *84.7* *	0.25 *53.2* *	45.1 *57.9* *
Big Muddy	org	43.0 *115.3*	20.0 *108.7*	8.5 *78.0*	5.9 *101.9*	2.30 *87.5* *	0.23 *57.2* *	42.7 *61.2* *
W. Yellow R.	org	34.5 *92.5*	17.0 *92.4*	4.0 *36.7* *	6.1 *99.2*	2.57 *97.9*	0.13 *101.1*	28.5 *91.6*
Mid.Fk.Salt R.	org	33.0 *88.5*	15.0 *81.5*	8.5 *78.0*	6.1 *98.2*	2.59 *98.6*	0.12 *112.2*	21.1 *123.7*

Table 5. Metric values for the three classes of sites using multihabitat data from Prairie streams, fall 1994. Differences in metric means between reference (ref), habitat degraded (hab) and organically enriched (org) sites were tested by t-test.

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Simpson	Shannon	Dominant
E.Fk.Grand R.	ref	34.5	17.0	13.5	6.3	0.10	2.82	15.6
Grindstone Cr.	ref	35.0	18.0	13.0	7.4	0.14	2.51	24.3
Marrowb Cr.	ref	35.5	17.0	9.5	6.5	0.14	2.63	30.3
No Cr.	ref	44.0	22.0	8.5	7.1	0.17	2.50	36.2
Spring Cr.	ref	37.5	18.0	10.0	6.7	0.12	2.67	24.1
MEAN		**37.3**	**18.4**	**10.9**	**6.8**	**0.13**	**2.62**	**26.1**
SD		**3.9**	**2.1**	**2.2**	**0.5**	**0.03**	**0.13**	**7.7**
Big Cr.	hab	35.0	14.0	9.0	7.8	0.28	2.15	16.8
Mussel Fk.	hab	36.0	16.0	8.5	7.5	0.12	2.63	22.8
N.Fk.Salt R.	hab	36.0	17.0	6.5	6.7	0.15	2.54	31.6
Wakenda Cr.	hab	33.5	15.0	5.0	8.0	0.16	2.26	26.8
MEAN		**35.1**	**15.5**	**7.3**	**7.5**	**0.17**	**2.39**	**24.5**
SD		**1.2**	**1.3**	**1.8**	**0.6**	**0.07**	**0.23**	**6.3**
Shoal Cr.	org	34.5	17.0	12.0	6.9	0.11	2.75	26.0
E.Fk.Big Cr.	org	39.5	18.0	8.5	7.1	0.25	2.22	45.1
Big Muddy	org	43.0	20.0	8.5	7.1	0.23	2.30	42.7
W. Yellow R.	org	34.5	17.0	4.0	7.8	0.13	2.57	28.5
Mid.Fk.Salt R.	org	33.0	15.0	8.5	7.2	0.12	2.59	21.1
MEAN		**36.9**	**17.4**	**8.3**	**7.2**	**0.17**	**2.48**	**32.7**
SD		**4.2**	**1.8**	**2.8**	**0.4**	**0.07**	**0.22**	**10.6**
t-test, p values								
REF/HAB		0.290	0.032	0.046	0.088	0.301	0.142	0.801
REF/ORG		0.897	0.429	0.159	0.114	0.360	0.244	0.310

overlap in box and whisker plots between REF and IMP streams. These plots indicate a median value and the box represents the 25th and 75th percentile of the values. Vertical lines from the box indicate the 10th and 90th percentiles of values (Fig. 3).

Metrics were judged to have one of four sensitivity values: a value of three if no overlap existed in the interquartile range, a sensitivity of two if some overlap occurred that did not extend to the medians, a sensitivity of one if there was some overlap of interquartile ranges but at least one median was outside the range, and a sensitivity of zero if interquartile overlap was considerable, with no discrimination between REF and IMP sites (after Barbour et al. 1992; see Chapter 6 for description of box plot analysis).

For streams in the Prairie region multihabitat data showed high sensitivity (values of 3) for the EPT (both HAB and ORG) and Family (HAB), and lesser sensitivity (values of 1 or 2) for the BI (HAB), Shannon's diversity index (HAB and ORG), and Simpson's diversity index (HAB) which distinguished both habitat degraded and organically distressed sites (Fig. 3, Table 1). The metrics Total taxa, BI, Family, and % Dominant taxon showed very little sensitivity for organically enriched situations.

Single Habitat Analysis

Many biologists prefer multihabitat analysis because more complete information is obtained (e.g., Lenat 1988). However, our common habitats in prairie streams consisted of just nonflow and fs flow. We determined that fs flow is not a productive habitat and produces metrics with considerable variation. Therefore, we reanalyzed the above data using just the nonflow habitat.

Community Structure

Ordination of the invertebrate community from a single habitat for Prairie region streams (Fig. 4) to compare REF vs. HAB showed better separation of site types than when using multihabitat data (Fig. 2). Communities from REF streams grouped closely together, except one stream, indicating good reference repeatability and with that same one exception, communities from REF sites did not intermingle with HAB sites. Ordination to compare REF communities vs. ORG communities was not as clear (Fig. 4). The two types of sites were not well separated.

Metric Sensitivity

We again compared REF to degraded conditions by examining which of the metric values from degraded streams fell outside the natural variation (CV) of REF sites (Table 6). There were many more differences than when using multihabitat data (Table 4). Every stream had at least one metric indicating IMP conditions. For HAB, the BI identified three IMP streams and % Dominant taxon identified only one, while the other metrics identified two of the four streams. For the ORG situation, EPT identified every site as degraded, BI and Shannon's diversity index identified two streams, while the remaining metrics identified three of the streams.

Statistical Analysis

A statistical analysis of differences of scores between types of streams indicated no significant differences for any metrics for REF-HAB comparisons (*t*-test, $P > 0.05$; Table 7). For the REF-ORG comparisons, only EPT and BI were significantly different ($P < 0.05$; Table 7).

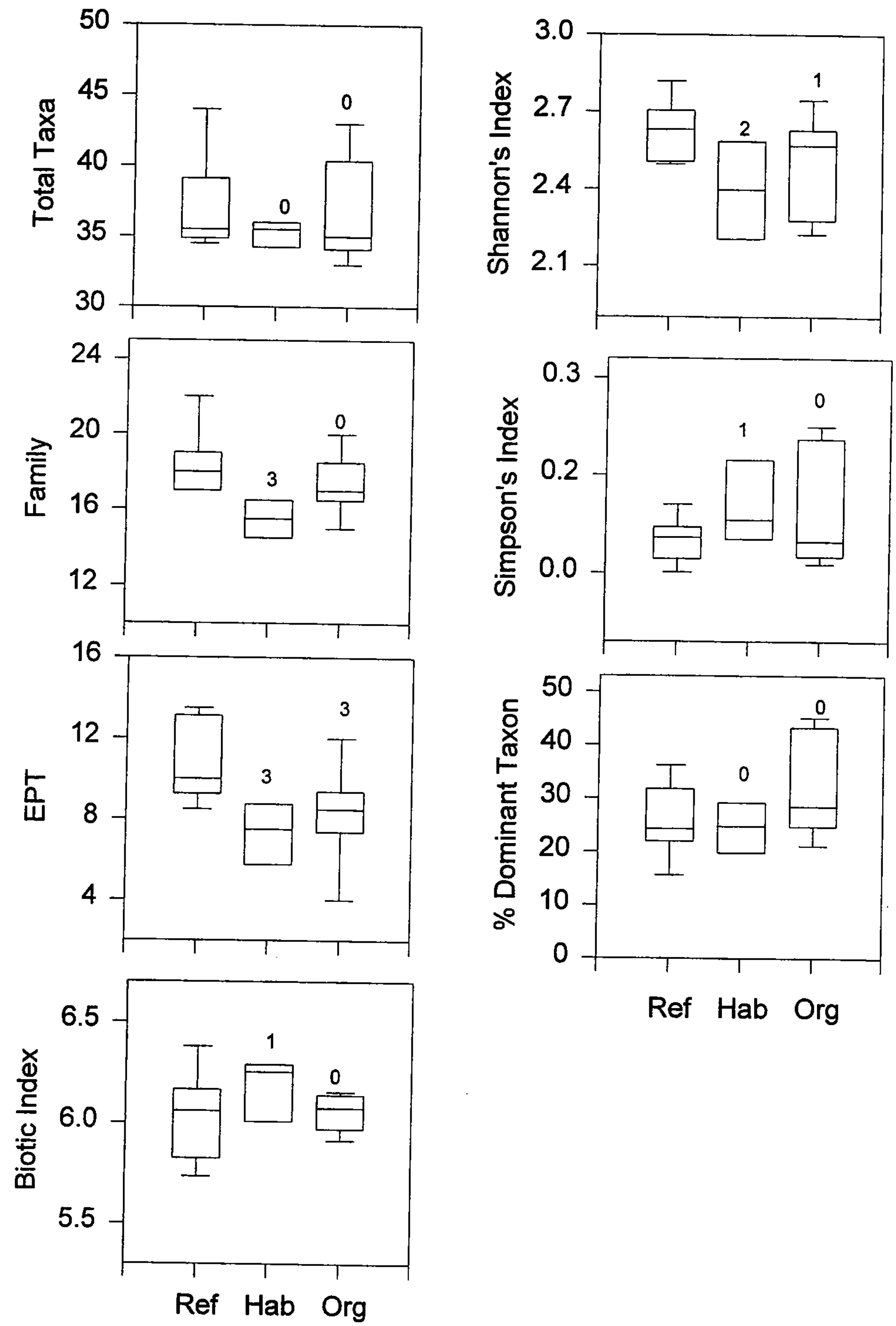


Fig. 3. Box and whisker plots comparing reference (REF), habitat degraded (HAB) and organically enriched (ORG) streams from the prairie ecoregion using multihabitat data, fall 1994. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

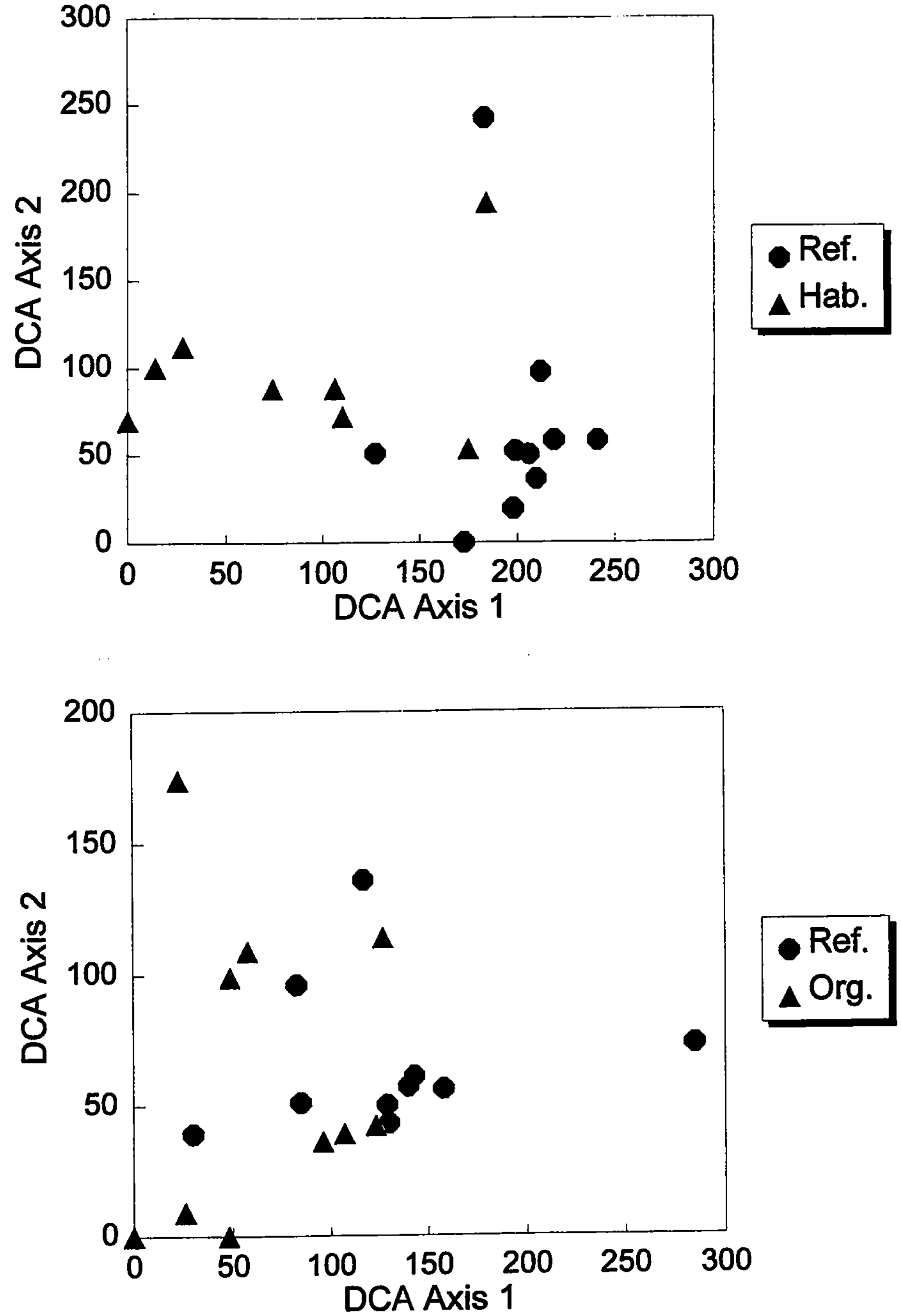


Fig. 4. Ordination of the reference (REF) and habitat degraded (HAB) sites, and the reference and organically enriched (ORG) sites, from the prairie ecoregion using single habitat data, fall 1994.

Table 6. Metric values for three classes of streams and metric means and C.V. of 5 reference streams for nonflow habitat samples from Prairie streams, fall 1994. Impairment thresholds are based on the C.V. values. Percentage metric changes (italic numbers) of a degraded stream to mean values of reference streams exceeding thresholds are marked with stars.

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
E.Fk.Grand R.	ref	27.0	14.0	10.0	6.4	2.53	0.14	29.0
Grindstone Cr	ref	27.5	14.0	9.0	7.4	2.34	0.17	32.6
Marrowb Cr.	ref	24.5	13.5	7.0	6.3	2.37	0.17	33.6
No Cr.	ref	26.0	15.0	6.5	7.3	2.23	0.18	33.0
Spring Cr.	ref	27.5	14.5	6.5	6.3	2.48	0.16	33.1
MEAN		26.5	14.2	7.8	6.7	2.39	0.16	32.3
C.V.		4.8	4.0	20.6	8.0	4.88	8.79	5.8
Impact Threshold		95%	95%	75%	90%	95%	90%	90%
Big Cr.	hab	29.0 *109.4*	14.5 *102.1*	9.0 *115.4*	7.9 *85.4* *	2.25 *94.1* *	0.23 *70.8* *	41.0 *78.9* *
Mussel Fk.	hab	23.5 *88.7* *	13.0 *91.5* *	6.5 *83.3*	7.5 *89.3* *	2.42 *101.4*	0.13 *127.8*	21.4 *151.3*
N.Fk.Salt R.	hab	23.5 *88.7* *	12.0 *84.5* *	4.0 *51.3* *	6.6 *102.3*	2.55 *106.6*	0.12 *140.0*	22.8 *142.0*
Wakenda Cr.	hab	29.0 *109.4*	14.0 *98.6*	3.5 *44.9* *	8.2 *82.0* *	2.09 *87.5* *	0.19 *85.0* *	31.8 *101.6*
Shoal Cr.	org	19.5 *73.6* *	11.0 *77.5* *	4.5 *57.7* *	7.5 *89.4* *	2.07 *86.7* *	0.23 *71.2* *	42.6 *75.8* *
E.Fk.Big Cr.	org	30.0 *113.2*	14.5 *102.1*	5.0 *64.1* *	7.2 *93.4*	2.12 *88.6* *	0.26 *62.5* *	47.8 *67.6* *
Big Muddy	org	32.0 *120.8*	15.5 *109.2*	5.0 *64.1* *	7.3 *92.7*	2.28 *95.3*	0.20 *81.9* *	37.6 *85.9* *
W. Yellow R.	org	23.0 *86.8* *	12.0 *84.5* *	2.5 *32.1* *	7.9 *85.5* *	2.39 *100.2*	0.15 *104.9*	31.3 *103.4*
Mid.Fk.Salt R.	org	23.0 *86.8* *	13.0 *91.5* *	5.0 *64.1* *	7.3 *92.0*	2.47 *103.5*	0.13 *123.8*	25.1 *128.9*

Table 7. Metric values for the three classes of sites using nonflow habitat data from Prairie streams, fall 1994. Differences in metric means between reference (ref), habitat degraded (hab) and organically enriched (org) sites were tested by t-test.

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
E.Fk.Grand R.	ref	27.0	14.0	10.0	6.4	2.53	0.14	29.0
Grindstone Cr.	ref	27.5	14.0	9.0	7.4	2.34	0.17	32.6
Marrowb Cr.	ref	24.5	13.5	7.0	6.3	2.37	0.17	33.6
No Cr.	ref	26.0	15.0	6.5	7.3	2.23	0.18	33.0
Spring Cr.	ref	27.5	14.5	6.5	6.3	2.48	0.16	33.1
MEAN		**26.5**	**14.2**	**7.8**	**6.7**	**2.39**	**0.16**	**32.3**
SD		**1.3**	**0.6**	**1.6**	**0.5**	**0.12**	**0.01**	**1.9**
Big Cr.	hab	29.0	14.5	9.0	7.9	2.25	0.23	41.0
Mussel Fk.	hab	23.5	13.0	6.5	7.5	2.42	0.13	21.4
N.Fk.Salt R.	hab	23.5	12.0	4.0	6.6	2.55	0.12	22.8
Wakenda Cr.	hab	29.0	14.0	3.5	8.2	2.09	0.19	31.8
MEAN		**26.3**	**13.4**	**5.8**	**7.6**	**2.33**	**0.16**	**29.2**
SD		**3.2**	**1.1**	**2.5**	**0.7**	**0.20**	**0.05**	**9.1**
Shoal Cr.	org	19.5	11.0	4.5	7.5	2.07	0.23	42.6
E.Fk.Big Cr.	org	30.0	14.5	5.0	7.2	2.12	0.26	47.8
Big Muddy	org	32.0	15.5	5.0	7.3	2.28	0.20	37.6
W. Yellow R.	org	23.0	12.0	2.5	7.9	2.39	0.15	31.3
Mid.Fk.Salt R.	org	23.0	13.0	5.0	7.3	2.47	0.13	25.1
MEAN		**25.5**	**13.2**	**4.4**	**7.4**	**2.27**	**0.19**	**36.9**
SD		**5.3**	**1.8**	**1.1**	**0.3**	**0.17**	**0.05**	**9.0**
t-test, p values								
REF/HAB		0.839	0.246	0.201	0.105	0.587	0.923	0.450
REF/ORG		0.581	0.250	0.007	0.030	0.219	0.286	0.378

Box and Whisker Plots

Single habitat (nonflow) prairie data had four metrics showing some sensitivity (Fig. 5), BI and EPT for both HAB and ORG, Shannon's diversity index for ORG, and Family for HAB. Only the EPT was similarly sensitive for both habitat and water quality degraded situations, regardless of the number of habitats used.

Prairie Region Conclusions

We had difficulty in consistently being able to differentiate between REF and "degraded" streams with many of the metrics. But some uncertainty exists because water sample and habitat scores, the DCA, and metric similarity comparisons all showed that some *a priori* selected IMP streams perhaps were not actually impacted. However, the REF stream CVs for all metrics again were low indicating some potential. Results of nonflow habitat alone were better than multihabitat data. There was some ambiguity in assessing the overall sensitivity of each metric, but generally EPT, BI, and Shannon's diversity index performed best, Family and Simpson's diversity index performed fairly well, while Total taxa and % Dominant taxon were least sensitive.

Ozark Streams

Multihabitat (cs flow + nonflow + rootmat)

Most sites had the three major habitat types: cs flow, nonflow, and rootmat (Table 8). Four sites did not have rootmat habitats. Earlier analysis indicated a close similarity between rootmat and vegetation communities, and vegetation was substituted for rootmats on these occasions.

Community Structure

When REF-HAB are compared on the ordination (Fig. 6), Spring River and Flat Creek separated from REF streams but the other degraded streams interspersed with REF sites. Unfortunately, this pattern of sites may have been related to water quality (higher nitrogen in Spring River and Flat Creek, rather than to only lower habitat scores [Table 9]). In the REF-ORG sites comparison (Fig. 6) REF sites were tightly organized and the majority of ORG sites were quite distinct. This pattern appears related to water quality (Table 9) because the interspersed sites had only slightly elevated nutrient levels, while the more dispersed ORG sites had levels orders of magnitude greater. Our *a priori* designation of impacted sites was probably not good in every instance.

Metric Sensitivity

The CVs of metric values within the REF group were all less than 15% except for Simpson's diversity index and % Dominant taxon which were still below 35% (Table 10). To compare REF to IMP we used mean variations of each metric from the REF and considered impairment when a value was outside the CV. For HAB sites only Spring River showed consistently impacted scores, only two other streams showed a single metric below threshold values. Even the Spring River result must be evaluated in light of its high nutrient levels (Table 9).

A much better discrimination was shown with the REF-ORG stream comparisons. All the metrics of the three definitely impacted sites (Turkey, Clear, and Dry creeks) showed very low similarity to REF conditions (Table 10).

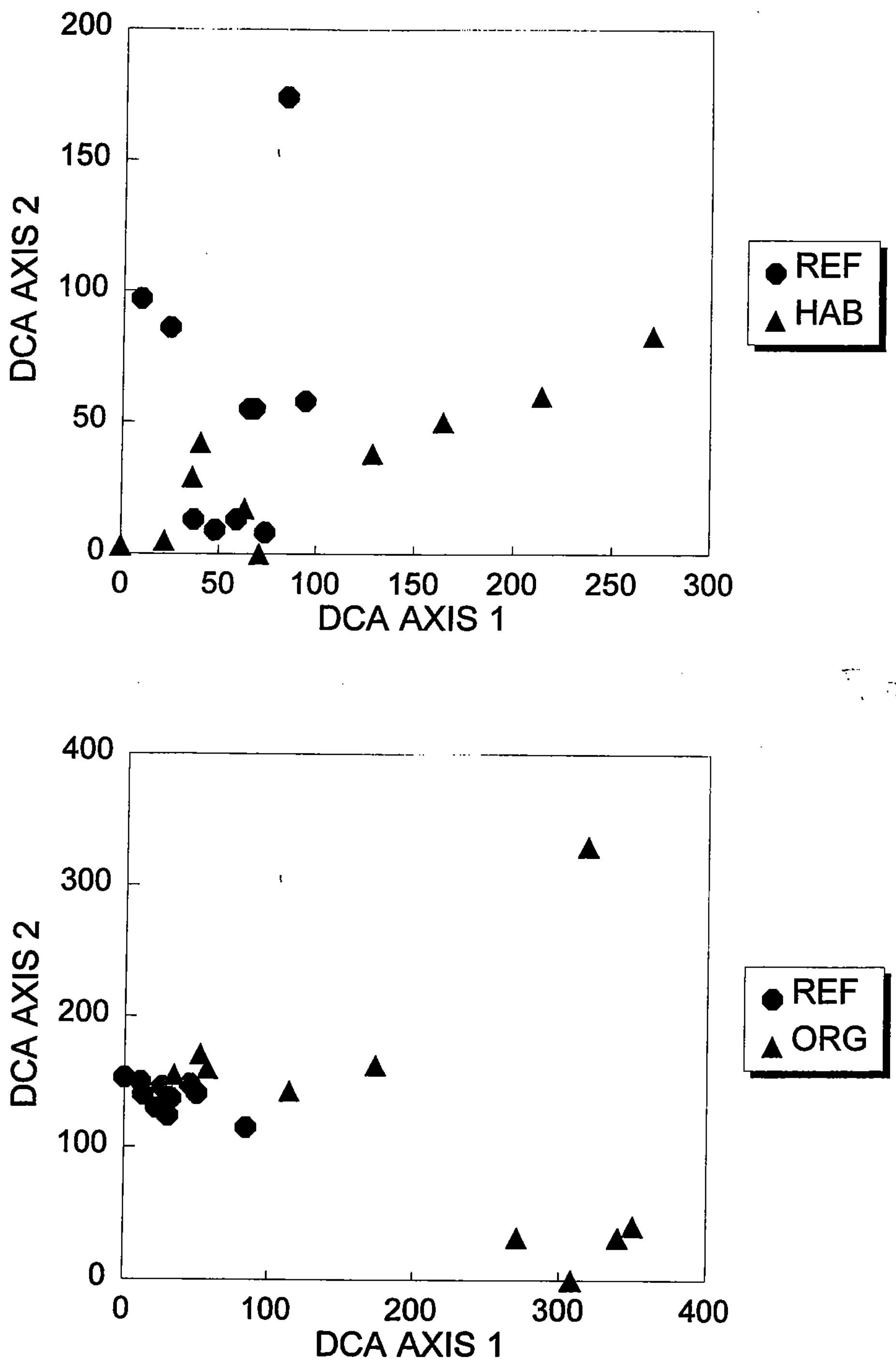


Fig. 6. Ordination of the reference (REF) and habitat degraded (HAB) sites, and the reference and organically enriched (ORG) sites, from the Ozark ecoregion using multihabitat data, fall 1994.

Table 9. Total nitrogen (TN, mg/L) and phosphorus (TP, ug/L), habitat scores for Ozark streams, Fall 1994

Stream	No.	TN			TP			SCORE		
		Site 1	Site 2	Mean	Site 1	Site 2	Mean	Site 1	Site 2	Mean
Reference streams										
Huzzah Cr.	28	0.12	0.11	0.12	5	4	5	144	150	147
Big Cr.	30	0.09	0.09	0.09	2	17	10	140	139	140
Bull Cr.	21	0.43	0.44	0.44	5	2	4	134	149	142
Big Sugar Cr.	17	0.62	1.00	0.81	14	26	20	151	136	144
Ltl Niangua	24	0.20	0.21	0.21	31	22	27	126	111	119
Habitat degraded streams										
IndianCr.	29	<LOD	0.13	0.07	5	12	9	109	107	108
Hutchins Cr.	27	0.09	<LOD	0.05	7	22	15	90	108	99
Woods Fk.	22	0.19	0.23	0.21	21	19	20	122	109	116
Flat Cr.	20	1.60	2.25	1.93	30	31	31	120	109	115
Spring R.	19	2.33	3.18	2.76	80	82	81	109	117	113
Organically enriched streams										
Spring Br.	26	1.98	1.28	1.63	106	81	94	131	146	139
Weststone Cr.	23	0.37	0.48	0.43	378	115	247	132	124	128
Turkey Cr.	16	7.04	5.63	6.34	1928	2582	2255	125	127	126
Clear Cr.	18	25.11	25.04	25.08	18285	14585	16435	122	110	116
Dry Auglaize	25	2.60	1.16	1.88	2909	2679	2794	103	103	103

Table 10. Metric values for three classes of streams and metic means and C.V. of 5 reference streams for multihabitat samples from Ozark streams, fall 1994. Impairment thresholds are based on the C.V. values. Percentage metric changes (italic numbers) of a degraded stream to mean values of reference streams exceeding thresholds are marked with stars.

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
Huzzah Cr.	ref	74.5	37.0	24.0	5.2	3.19	0.08	19.5
Big Cr.	ref	64.0	33.0	18.0	5.7	3.05	0.11	27.7
Bull Cr.	ref	53.5	32.0	18.5	4.2	2.88	0.15	35.3
Big Sugar Cr.	ref	60.0	33.0	22.5	4.3	2.97	0.11	25.8
Ltl Niangua	ref	55.5	33.0	18.5	5.8	3.32	0.06	15.0
Mean		61.5	34.0	20.3	5.1	3.08	0.10	24.7
C.V.		13.5	5.8	13.6	15.5	5.6	32.2	31.7
Impact threshold		85%	90%	85%	80%	85%	65%	65%
IndianCr.	hab	66.0 107.3	36.0 105.9	22.0 108.4	5.6 90.5	2.83 91.7	0.15 66.7	33.5 73.7
Hutchins Cr.	hab	59.5 96.7	33.0 97.1	20.5 101.0	5.3 94.7	2.87 93.0	0.14 74.1	31.6 78.2
Woods Fk.	hab	60.5 98.4	29.0 85.3	19.0 93.6	5.8 87.4	2.92 94.8	0.10 105.3	21.6 114.4
Flat Cr.	hab	63.5 103.3	35.0 102.9	22.0 108.4	5.8 86.5	3.37 109.4	0.06 181.8	14.4 171.5
Spring R.	hab	39.0 63.4 *	21.0 61.8 *	14.0 69.0 *	6.2 80.9	2.75 89.1	0.11 95.2	23.8 103.8
Spring Br.	org	54.5 88.6	28.0 82.4 *	12.0 59.1 *	6.3 80.0	3.28 106.5	0.06 181.8	10.2 242.2
Weststone Cr.	org	64.0 104.1	30.0 88.2 *	19.0 93.6	5.6 89.7	3.13 101.5	0.07 142.9	19.5 126.7
Turkey Cr.	org	26.0 42.3 *	18.0 52.9 *	2.0 9.9 *	9.1 55.4 *	1.59 51.5 *	0.40 25.3 *	61.0 40.5 *
Clear Cr.	org	26.0 42.3 *	13.0 38.2 *	0.5 2.5 *	8.1 62.0 *	2.15 69.8 *	0.19 52.6 *	36.2 68.2
Dry Auglaize	org	30.5 49.6 *	21.0 61.8 *	3.5 17.2 *	8.2 61.8 *	1.97 64.0 *	0.29 34.5 *	43.6 56.7 *

Statistical Analysis

A comparison of the mean values for each metric showed no significant differences between REF and HAB (Table 11), although the BI was marginal at P = 0.102. The comparison of metrics between REF and ORG streams shows significant differences for Total taxa, Family, EPT, and BI, with Shannon's diversity index value marginal at P = 0.085.

There was high variation within the ORG group because two of the five sites had much better scores for every metric (Table 11) which is consistent with our water quality data (Table 9), the ordination, and the metric similarity comparison (Table 10).

Based on these results we can say with some confidence that these three streams—Turkey, Clear, and Dry Auglaize creeks—were impaired and sensitive metrics should have, and did, detect the impairment. When we compare mean values between REF and the three impacted ORG sites we found all seven metrics were significantly different ($p < 0.05$; Table 11).

Box and Whisker Plots

Ozark streams had more metrics that showed good sensitivity than was the case for prairie streams (Fig. 7). For the multihabitat analysis, water quality degradation (ORG) was readily detected by all but the Simpson's diversity index and % Dominant taxon metrics. Habitat degraded situations were less often distinguished, although the BI and Shannon's diversity index showed sensitivities of 1 and 2, respectively.

Conclusion

Metrics often failed to detect habitat degradation, but were sensitive to water quality degradation. Some of the ambiguity may stem from our *a priori* selection of impacted sites which turned out not to be so.

Single Habitat Evaluation

The single habitat cs flow (i.e., riffle–run) is recommended for developing a Rapid Bioassessment Protocol (Plafkin et al. 1989). The cs flow is a common habitat in streams of the Ozark region and was chosen here to be evaluated and compared with multihabitat data.

Community Structure

Ordination using REF-HAB sites (Fig. 8) produced similar results to multihabitat data (Fig. 6). Sites did not separate well, and distinct clusters of stream types were not evident. The REF–ORG sites plot (Fig. 8) was also similar to multihabitat (Fig. 6), where good separation between the two stream types was evident. Degraded sites dispersed widely, with the most enriched sites being furthest from the reference groupings.

Metric Sensitivity

Results of examining metrics for a departure from the natural variation (Table 12) for the single habitat were quite similar to those for multihabitat (Table 10), with two notable differences. First, the cs flow result showed how two HAB streams, Indian and Hutchin's creeks, were well distinguished by the two diversity metrics and % Dominant taxon (Table 12). This suggests that diversity metrics may have utility for detecting habitat problems. Secondly, higher % Dominant taxon made two highly enriched streams "unimpacted." Overall, every stream but one was classed as impacted by at least one metric. The mean percentage of metrics that showed

Table 11. Metric values for the three classes of sites using multihabitat data from Ozark streams, fall 1994. Differences in metric means between reference (ref), habitat degraded (hab) and organically enriched (org) sites were tested by t-test.

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
Huzzah Cr.	ref	74.5	37.0	24.0	5.2	3.19	0.08	19.5
Big Cr.	ref	64.0	33.0	18.0	5.7	3.05	0.11	27.7
Bull Cr.	ref	53.5	32.0	18.5	4.2	2.88	0.15	35.3
Big Sugar Cr.	ref	60.0	33.0	22.5	4.3	2.97	0.11	25.8
Ltl Niangua	ref	55.5	33.0	18.5	5.8	3.32	0.06	15.0
Mean		**61.5**	**33.6**	**20.3**	**5.1**	**3.08**	**0.10**	**24.7**
SD		**8.3**	**1.9**	**2.8**	**0.8**	**0.17**	**0.03**	**7.8**
IndianCr.	hab	66.0	36.0	22.0	5.6	2.83	0.15	33.5
Hutchins Cr.	hab	59.5	33.0	20.5	5.3	2.87	0.14	31.6
Woods Fk.	hab	60.5	29.0	19.0	5.8	2.92	0.10	21.6
Flat Cr.	hab	63.5	35.0	22.0	5.8	3.37	0.06	14.4
Spring R.	hab	39.0	21.0	14.0	6.2	2.75	0.11	23.8
Mean		**57.7**	**30.8**	**19.5**	**5.8**	**2.95**	**0.11**	**25.0**
SD		**10.8**	**6.1**	**3.3**	**0.3**	**0.25**	**0.04**	**7.8**
Spring Br.	org	54.5	28.0	12.0	6.3	3.28	0.06	10.2
Weststone Cr.	org	64.0	30.0	19.0	5.6	3.13	0.07	19.5
Turkey Cr.	org	26.0	18.0	2.0	9.1	1.59	0.40	61.0
Clear Cr.	org	26.0	13.0	0.5	8.1	2.15	0.19	36.2
Dry Auglaize	org	30.5	21.0	3.5	8.2	1.97	0.29	43.6
Mean		**40.2**	**22.0**	**7.4**	**7.5**	**2.42**	**0.20**	**34.1**
SD		**17.8**	**7.0**	**7.9**	**1.4**	**0.74**	**0.15**	**20.0**
t-test, p values								
Ref/Hab		0.531	0.337	0.664	0.102	0.321	0.786	0.956
Ref/Org		0.039	0.016	0.034	0.010	0.085	0.293	0.600
Ref/3 high Org		0.000	0.037	0.045	0.001	0.026	0.015	0.025

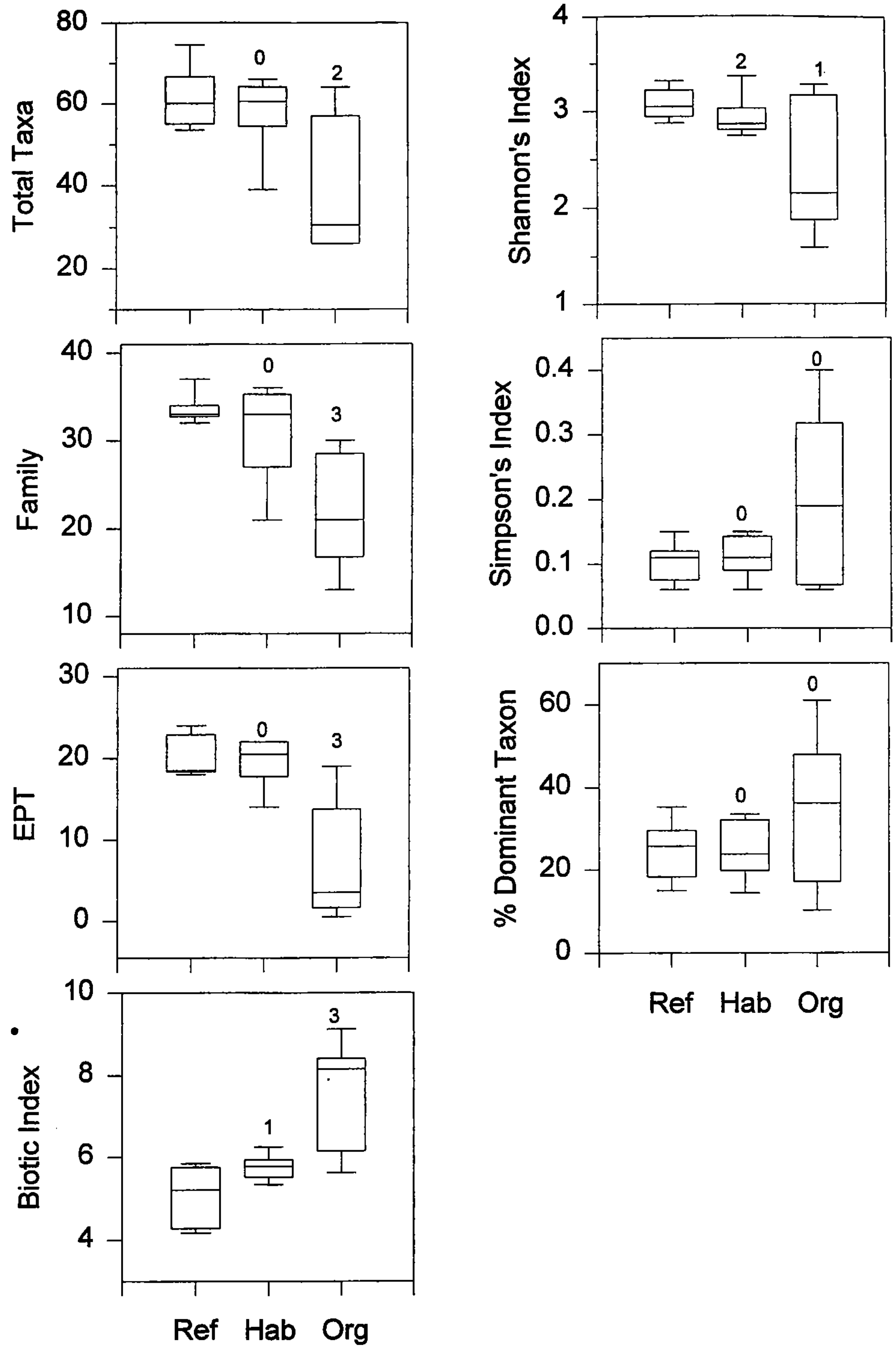


Fig. 7. Box and whisker plots comparing reference (REF), habitat degraded (HAB) and organically enriched (ORG) streams from the Ozark ecoregion using multihabitat data, fall 1994. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

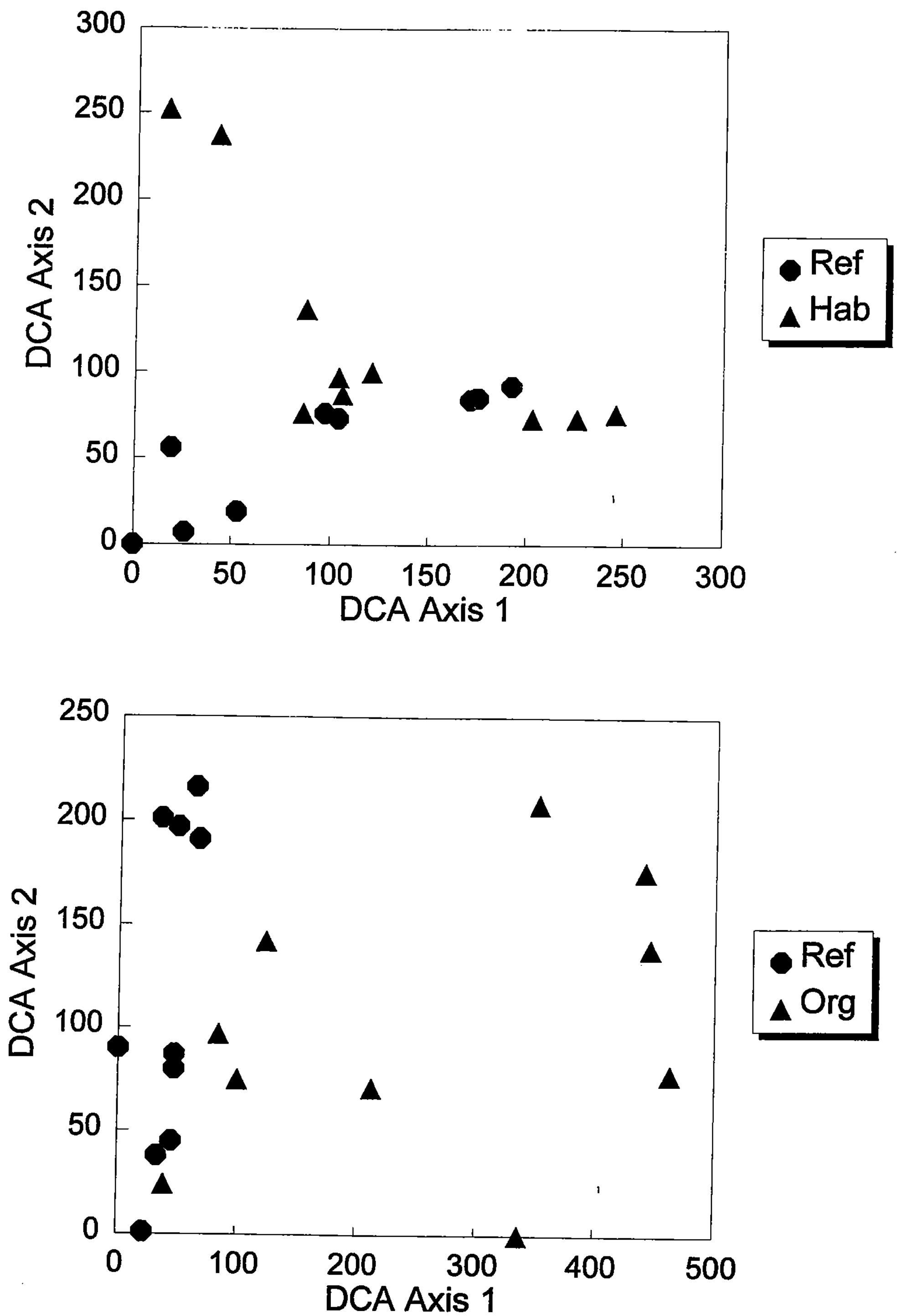


Fig. 8. Ordination of the reference (REF) and habitat degraded (HAB) sites, and the reference and organically enriched (ORG) sites, from the Ozark ecoregion using single habitat data, fall 1994.

Table 12. Metric values for three classes of streams and metric means and C.V. of 5 reference streams for cs flow habitat samples from Ozark streams, fall 1994. Impairment thresholds are based on the C.V. values. Percentage metric changes (italic numbers) of a degraded stream to mean values of reference streams exceeding thresholds are marked with stars.

Stream	Class	Taxa	Family	EPT	Biotic Ind	Shannon	Simpson	Dominant
Huzzah Cr.	ref	41.0	22.0	15.0	4.9	2.72	0.13	29.15
Big Cr.	ref	24.5	17.5	11.5	5.0	2.08	0.25	46.80
Bull Cr.	ref	28.5	19.0	14.5	3.6	2.40	0.19	40.50
Big Sugar Cr.	ref	24.0	15.5	12.5	3.3	2.25	0.20	40.10
Ltl Niangua	ref	23.5	17.0	12.0	4.5	2.72	0.09	16.55
Mean		28.3	18.2	13.1	4.3	2.43	0.17	34.6
C.V.		26.0	13.5	11.9	18.0	10.5	37.9	30.8
Impact threshold		70%	85%	85%	80%	85%	60%	70%
IndianCr.	hab	25.5 *90.1*	16.5 *90.7*	12.5 *95.4*	4.9 *87.0*	1.99 *81.8* *	0.31 *55.0* *	48.35 *71.6*
Hutchins Cr.	hab	31.5 *111.3*	20.0 *109.9*	15.5 *118.3*	4.9 *86.9*	2.03 *83.6* *	0.30 *57.8* *	52.45 *66.0* *
Woods Fk.	hab	30.5 *107.8*	17.5 *96.2*	16.0 *122.1*	5.4 *79.5* *	2.51 *103.1*	0.15 *111.9*	33.15 *104.4*
Flat Cr.	hab	33.0 *116.6*	21.5 *118.1*	16.5 *138.8*	5.4 *79.0* *	2.69 *110.4*	0.09 *201.5*	13.10 *264.3*
Spring R.	hab	18.5 *65.4* *	12.0 *65.9* *	9.5 *72.5* *	5.1 *84.2*	2.32 *95.1*	0.13 *128.6*	20.70 *167.2*
Spring Br.	org	23.0 *81.3*	12.0 *65.9* *	7.0 *53.4* *	5.7 *75.2* *	2.48 *101.7*	0.13 *136.7*	26.05 *132.9*
Whetstone Cr.	org	33.0 *116.6*	19.0 *104.4*	13.0 *99.2*	5.1 *84.2*	2.70 *111.0*	0.10 *170.6*	19.20 *180.3*
Turkey Cr.	org	10.5 *37.1* *	6.0 *33.0* *	0.5 *3.8* *	8.6 *50.0* *	1.39 *57.0* *	0.34 *50.1* *	50.25 *68.9* *
Clear Cr.	org	16.5 *58.3* *	8.0 *44.0* *	0.5 *3.8* *	7.3 *58.5* *	1.86 *76.4* *	0.23 *74.6*	36.95 *93.7*
Dry Auglaize	org	14.0 *49.5* *	10.0 *54.9* *	2.5 *19.1* *	7.4 *58.0* *	1.87 *76.7* *	0.25 *68.6*	44.50 *77.8*

impairment for any one stream was 28 for HAB streams and 71 for ORG streams.

Statistical Analysis

Total taxa richness (Table 13) was lower and % Dominant taxon higher than the values obtained from using multihabitat data (Table 11); however, results of testing metric sensitivity were similar to those from multihabitat data, except there were no significant differences in Total taxa between REF and HAB streams (Table 13).

Box and Whisker plots

Similar discrimination was shown for the single habitat HAB comparisons as for the multihabitat comparisons. REF-ORG differences were greatest for the Total taxa, Family, EPT, and BI, while REF-HAB distinctions were only shown for the BI (Fig. 9).

Evaluation of Definitely Impaired Ozark Streams

An analysis of water quality and habitat scores from the fall 1994 sites showed an obvious impairment of four streams (Table 9), with both water quality and habitat problems. Our evaluation of numerous candidate streams in Missouri indicated that a multiple–impacted stream is the more common situation than either an ORG or a HAB site. This analysis is between REF conditions and four obviously impaired sites: Spring River and Turkey, Clear, and Dry Auglaize creeks.

Multihabitat (cs flow + nonflow + rootmats)

Community Structure

The DCA clearly separated REF from IMP sites (Fig. 10). REF sites were tightly grouped together, indicating high similarity. IMP sites were more dispersed, but all were distinct from the REF.

Metric Similarity

Variation among REF streams metric values was typically very low except for Simpson's diversity index and % Dominant taxon (Table 14). All metrics from IMP streams showed values below the impact threshold. The only exception was Simpson's diversity index and % Dominant taxon for a single stream.

Statistical Analysis

Seven metrics were calculated for each site (Table 15). Significant differences ($p < 0.05$) between REF and IMP were found for Total taxa, Family, EPT, BI, and Shannon's diversity index. Simpson's diversity index and % Dominant taxon were marginally significant ($p < 0.010$).

Box and Whisker Plots

All seven metrics showed no interquartile overlap (Fig. 11) indicating their strong ability to discriminate between REF and IMP.

Single Habitat (cs flow)

Community Structure

REF sites grouped together strongly and separated themselves from the IMP (Fig. 12). IMP sites were much more dispersed. Overall the distinction between IMP and REF was about the same whether multiple or single habitats were used.

Metric Similarity

Total taxa, Family, and EPT discriminated REF from IMP for every stream (Table 16). The BI and Shannon's

Table 13. Metric values for the three classes of sites using cs flow habitat data from Ozark streams, fall 1994. Differences in metric means between reference (ref), habitat degraded (hab) and organically enriched (org) sites were tested by t-test.

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
Huzzah Cr.	ref	41.0	22.0	15.0	4.9	2.72	0.13	29.2
Big Cr.	ref	24.5	17.5	11.5	5.0	2.08	0.25	46.8
Bull Cr.	ref	28.5	19.0	14.5	3.6	2.40	0.19	40.5
Big Sugar Cr.	ref	24.0	15.5	12.5	3.3	2.25	0.20	40.1
Ltl Niangua	ref	23.5	17.0	12.0	4.5	2.72	0.09	16.6
Mean		**28.3**	**18.2**	**13.1**	**4.3**	**2.43**	**0.17**	**34.6**
SD		**6.6**	**2.2**	**1.4**	**0.7**	**0.25**	**0.06**	**10.7**
IndianCr.	hab	25.5	16.5	12.5	4.9	1.99	0.31	48.4
Hutchins Cr.	hab	31.5	20.0	15.5	4.9	2.03	0.30	52.5
Woods Fk.	hab	30.5	17.5	16.0	5.4	2.51	0.15	33.2
Flat Cr.	hab	33.0	21.5	16.5	5.4	2.69	0.09	13.1
Spring R.	hab	18.5	12.0	9.5	5.1	2.32	0.13	20.7
Mean		**27.8**	**17.5**	**14.0**	**5.1**	**2.31**	**0.20**	**33.6**
SD		**5.3**	**3.3**	**2.6**	**0.2**	**0.27**	**0.09**	**15.2**
Spring Br.	org	23.0	12.0	7.0	5.7	2.48	0.13	26.1
Weststone Cr.	org	33.0	19.0	13.0	5.1	2.70	0.10	19.2
Turkey Cr.	org	10.5	6.0	0.5	8.6	1.39	0.34	50.3
Clear Cr.	org	16.5	8.0	0.5	7.3	1.86	0.23	37.0
Dry Auglaize	org	14.0	10.0	2.5	7.4	1.87	0.25	44.5
Mean		**19.4**	**11.0**	**4.7**	**6.8**	**2.06**	**0.21**	**35.4**
SD		**7.9**	**4.5**	**4.8**	**1.3**	**0.47**	**0.09**	**11.5**
t-test, p values								
Ref/Hab		0.921	0.671	0.669	0.052	0.507	0.797	0.775
Ref/Org		0.089	0.022	0.030	0.007	0.182	0.583	0.928
Ref/3 high Org		0.010	0.018	0.036	0.001	0.045	0.061	0.214

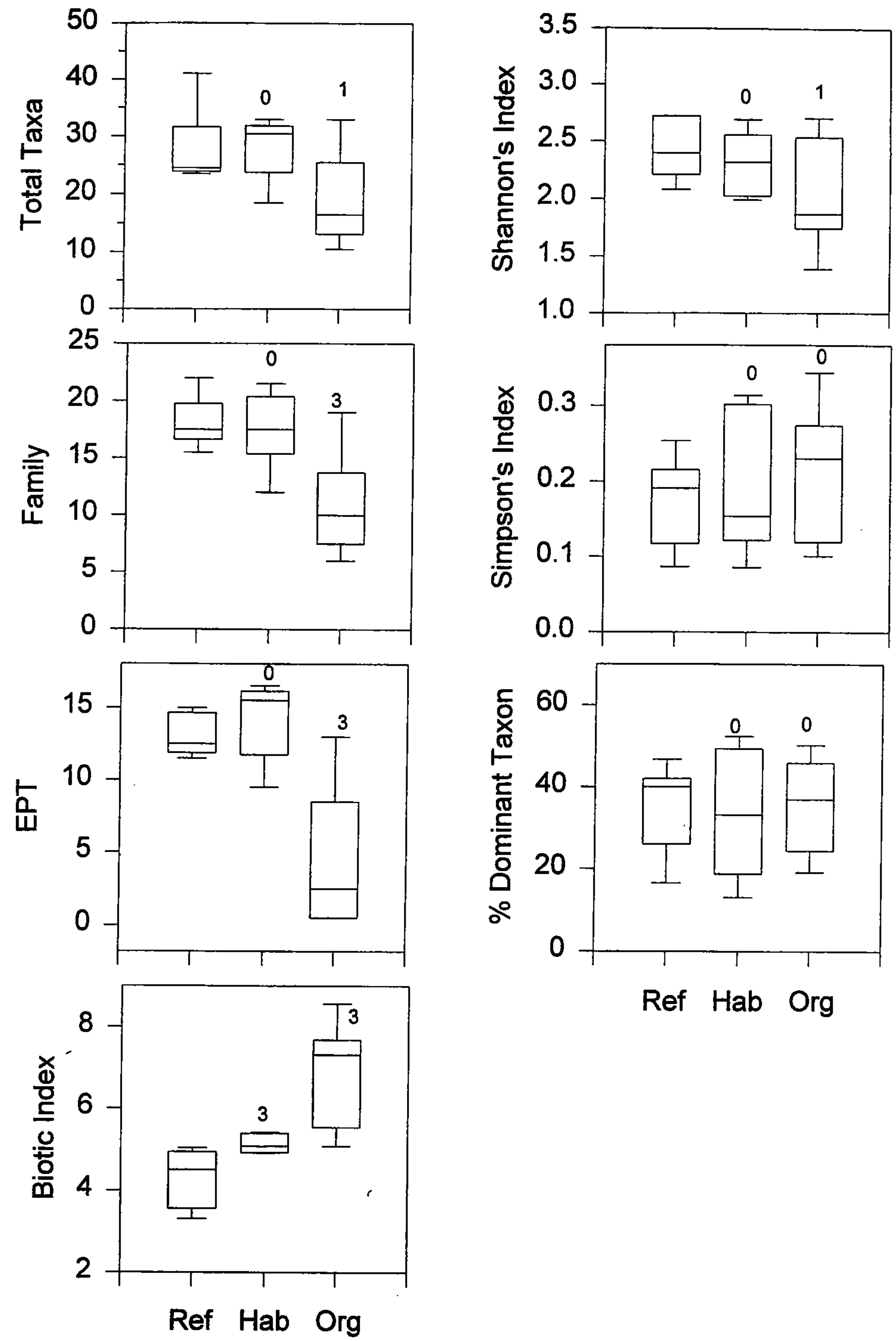


Fig. 9. Box and whisker plots comparing reference (REF), habitat degraded (HAB) and organically enriched (ORG) streams from the Ozark ecoregion using single habitat data, fall 1994. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

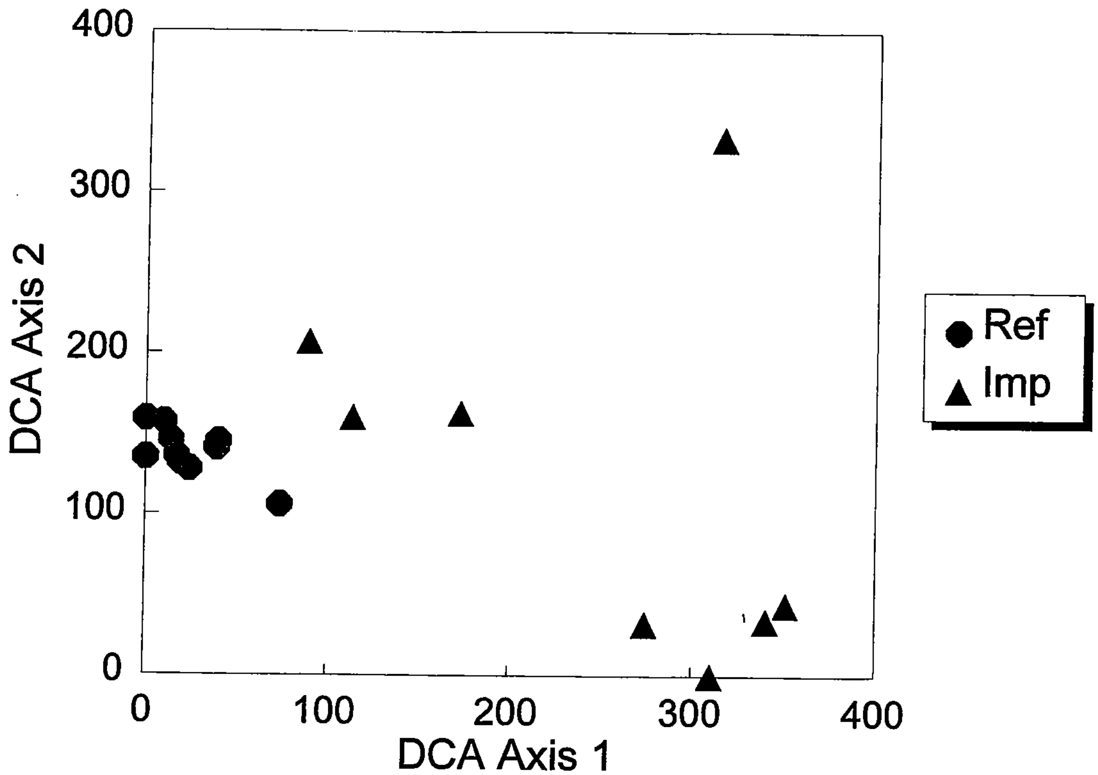


Fig. 10. Ordination of the reference (REF) and impaired (IMP) sites using multihabitat data, fall 1994.

Table 14. Metric values for reference and impaired streams and metric means and C.V. of 5 reference streams for multihabitat samples from Ozark streams, fall 1994. Impairment thresholds are based on the C.V. values. Percentage metric change (italic numbers) of a impaired stream to mean values of reference streams exceeding thresholds are marked with a star.

Stream	Class	Taxa		Family		EPT		Biotic Ind.		Shannon		Simpson		Dominant	
Huzzah Cr.	ref	74.5		37.0		24.0		5.2		3.19		0.08		19.5	
Big Cr.	ref	64.0		33.0		18.0		5.7		3.05		0.11		27.7	
Bull Cr.	ref	53.5		32.0		18.5		4.2		2.88		0.15		35.3	
Big Sugar Cr	ref	60.0		33.0		22.5		4.3		2.97		0.11		25.8	
Ltl Niangua	ref	55.5		33.0		18.5		5.8		3.32		0.06		15.0	
Mean		61.5		34.0		20.3		5.1		3.08		0.10		24.7	
C.V.		13.5		5.8		13.6		15.5		5.6		32.2		31.7	
Impact threshold		85%		90%		85%		85%		90%		65%		65%	
Spring R.	imp	39.0	*63.4* *	21.0	*61.8* *	14.0	*69.0* *	6.2	*80.9* *	2.75	*89.1* *	0.11	*95.2*	23.8	*103.8*
Turkey Cr.	imp	26.0	*42.3* *	18.0	*52.9* *	2.0	*9.9* *	9.1	*55.4* *	1.59	*51.5* *	0.40	*25.3* *	61.0	*40.5* *
Clear Cr.	imp	26.0	*42.3* *	13.0	*38.2* *	0.5	*2.5* *	8.1	*62.0* *	2.15	*69.8* *	0.19	*52.6* *	36.2	*68.2* *
Dry Auglaize	imp	30.5	*49.6* *	21.0	*61.8* *	3.5	*17.2* *	8.2	*61.8* *	1.97	*64.0* *	0.29	*34.5* *	43.6	*56.7* *

Table 15. Metric values for the three classes of sites using multihabitat data from Ozark streams, fall 1994. Differences in metric means between reference (ref) and definitely impaired (imp) sites were tested by t-test

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
Huzzah Cr.	ref	74.5	37.0	24.0	5.2	3.19	0.08	19.5
Big Cr.	ref	64.0	33.0	18.0	5.7	3.05	0.11	27.7
Bull Cr.	ref	53.5	32.0	18.5	4.2	2.88	0.15	35.3
Big Sugar Cr.	ref	60.0	33.0	22.5	4.3	2.97	0.11	25.8
Ltl Niangua	ref	55.5	33.0	18.5	5.8	3.32	0.06	15.0
Mean		**61.5**	**33.6**	**20.3**	**5.1**	**3.08**	**0.10**	**24.7**
SD		**8.3**	**1.9**	**2.8**	**0.8**	**0.17**	**0.03**	**7.8**
Spring R.	imp	39.0	21.0	14.0	6.2	2.75	0.11	23.8
Turkey Cr.	imp	26.0	18.0	2.0	9.1	1.59	0.40	61.0
Clear Cr.	imp	26.0	13.0	1.0	8.1	2.15	0.19	36.2
Dry Auglaize	imp	30.5	21.0	3.5	8.2	1.97	0.29	43.6
Mean		**30.4**	**18.3**	**5.1**	**7.9**	**2.11**	**0.25**	**41.2**
SD		**6.1**	**3.8**	**6.0**	**1.2**	**0.48**	**0.13**	**15.6**
t-test, p values								
Ref/Imp		0.001	0.010	0.044	0.005	0.037	0.055	0.089

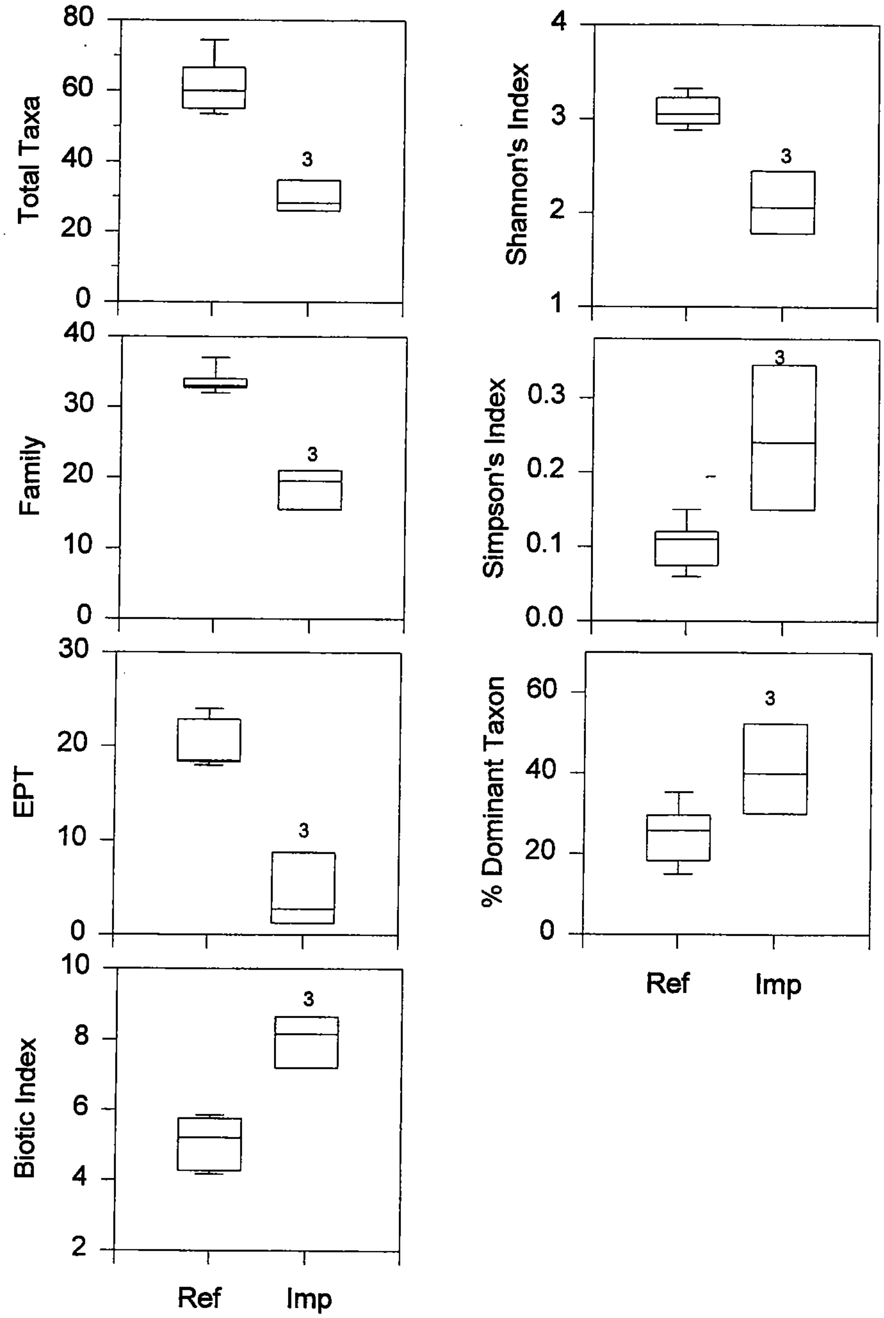


Fig 11. Box and whisker plots comparing reference (REF) and impaired (IMP) streams using multihabitat data, fall 1994. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

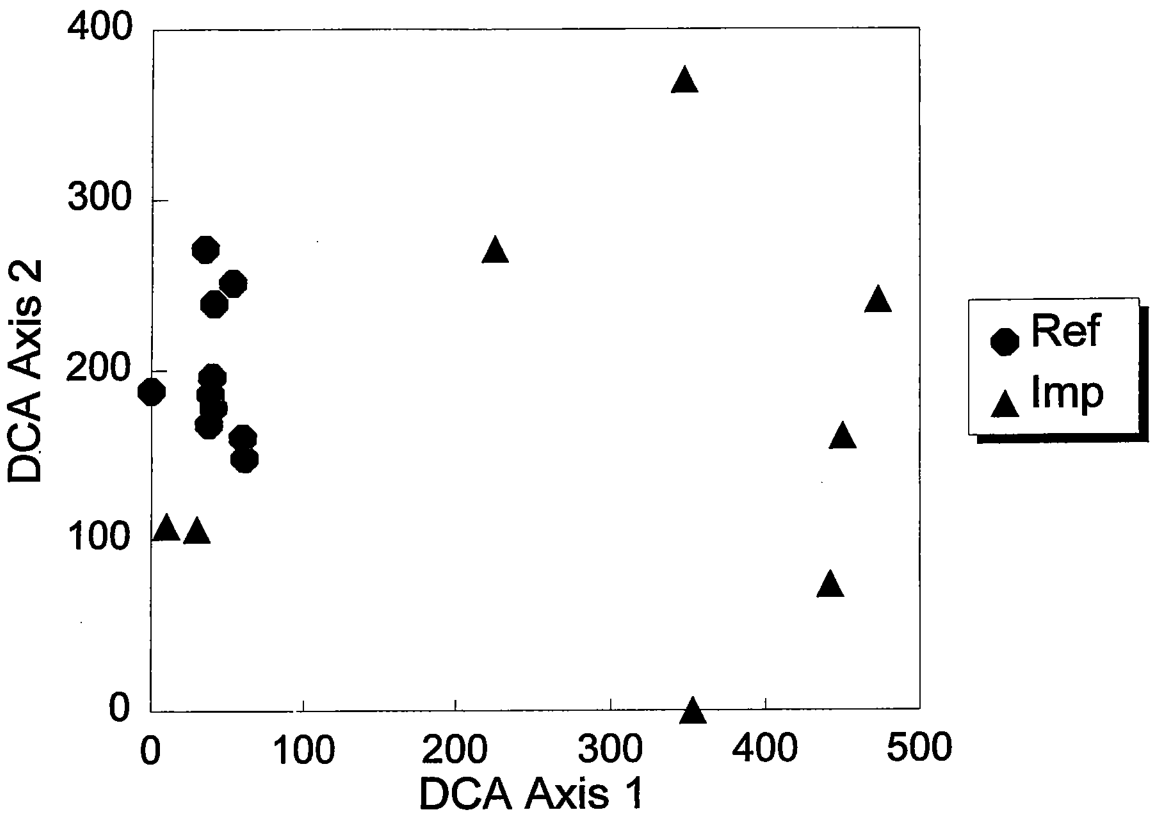


Fig. 12. Ordination of the reference (REF) and impaired (IMP) sites using single habitat data, fall 1994.

Table 16. Metric values for reference and impaired streams and metric means and C.V. of 5 reference streams for cs flow habitat samples from Ozark streams, fall 1994. Impairment thresholds are based on the C.V. values. Percentage metric change (italic numbers) of a impaired stream to mean values of reference streams exceeding thresholds are marked with a star.

Stream	Class	Taxa		Family		EPT		Biotic Ind.		Shannon		Simpson		Dominant	
Huzzah Cr.	ref	41.0		22.0		15.0		4.9		2.72		0.13		29.15	
Big Cr.	ref	24.5		17.5		11.5		5.0		2.08		0.25		46.80	
Bull Cr.	ref	28.5		19.0		14.5		3.6		2.40		0.19		40.50	
Big Sugar Cr	ref	24.0		15.5		12.5		3.3		2.25		0.20		40.10	
Ltl Niangua	ref	23.5		17.0		12.0		4.5		2.72		0.09		16.55	
Mean		28.3		18.2		13.1		4.3		2.43		0.17		34.6	
C.V.		26.0		13.5		11.9		18.0		11.7		37.9		34.5	
Impact threshold		75%		85%		85%		80%		85%		65%		65%	
Spring R.	imp	18.5	*65.4* *	12.0	*65.9* *	9.5	*72.5* *	5.1	*84.2*	2.32	*95.1*	0.13	*128.6*	20.70	*167.2*
Turkey Cr.	imp	10.5	*37.1* *	6.0	*33.0* *	0.5	*3.8* *	8.6	*50.0* *	1.39	*57.0* *	0.34	*50.1* *	50.25	*68.9*
Clear Cr.	imp	16.5	*58.3* *	8.0	*44.0* *	0.5	*3.8* *	7.3	*58.5* *	1.86	*76.4* *	0.23	*74.6*	36.95	*93.7*
Dry Auglaize	imp	14.0	*49.5* *	10.0	*54.9* *	2.5	*19.1* *	7.4	*58.0* *	1.87	*76.7* *	0.25	*68.6*	44.50	*77.8*

diversity index discriminated three of the four IMP streams, while Simpson's diversity index only discriminated one stream, and % Dominant taxon did none. These results were very comparable to the multihabitat analysis.

Statistical Analysis

Results were identical to those of multihabitat data, where Total taxa, Family, EPT, BI, and Shannon's diversity index showed significant ($p < 0.05$) or marginally significant ($p < 0.10$) differences between REF and IMP sites, while Simpson's diversity index and % Dominant taxon did not (Table 17).

Box and Whisker Plots

The same five metrics that showed no interquartile overlap with multihabitat data: Total taxa, Family, EPT, BI, and Shannon's diversity index also showed no quartile overlap between IMP and REF using single habitat data (Fig. 13). Single habitat data was not as good at discriminating impairment as was multihabitat data when using the metrics % Dominant taxon and Simpson's diversity index.

Conclusions for Ozark Streams

Our sequence of analyses provides consistent repeatable results. That is, if community structure, as shown by ordination plots, showed distinguishable grouping between REF and either HAB or ORG then either or both the statistical or similarity evaluation showed differences in metrics. If sites were interspersed on the ordination, indicating no discernible differences among REF and HAB or ORG, then metrics were not be able to indicate IMP conditions. Five of the metrics were shown to be excellent at detecting degradation: Total taxa, Family, EPT, BI and Shannon's diversity index. Such good discrimination using so few REF sites appeared to be due to low variation among REF sites. This again emphasizes the importance of REF site selection.

Part A Conclusion

Analyses of the fall 1994 dataset indicated the ability of our methods to detect both moderate and severe enrichment in both Prairie region streams and Ozark region streams. Specifically 1) degraded situations in the Ozark region are more readily observable than those in the prairie; 2) organically affected streams are readily discernible from REF streams by most of the metrics; 3) habitat degraded sites were not as readily detected by most metrics—while there was more difficulty in detecting habitat degraded streams, the two diversity indices and % Dominant taxon were most sensitive; 4) overall, there was about equal sensitivity using multi- or single habitat analysis.

Table 17. Metric values for the three classes of sites using cs flow habitat data from Ozark streams, fall 199 Differences in metric means between reference (ref) and definitely impaired (imp) sites were tested by t-test

Stream	Class	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
Huzzah Cr.	ref	41.0	22.0	15.0	4.9	2.72	0.13	29.2
Big Cr.	ref	24.5	17.5	11.5	5.0	2.08	0.25	46.8
Bull Cr.	ref	28.5	19.0	14.5	3.6	2.40	0.19	40.5
Big Sugar Cr.	ref	24.0	15.5	12.5	3.3	2.25	0.20	40.1
Ltl Niangua	ref	23.5	17.0	12.0	4.5	2.72	0.09	16.6
Mean		**28.3**	**18.2**	**13.1**	**4.3**	**2.43**	**0.17**	**34.6**
SD		**7.4**	**2.5**	**1.6**	**0.8**	**0.29**	**0.07**	**11.9**
Spring R.	imp	18.5	12.0	9.5	5.1	2.32	0.13	20.7
Turkey Cr.	imp	10.5	6.0	1.0	8.6	1.39	0.34	50.3
Clear Cr.	imp	16.5	8.0	1.0	7.3	1.86	0.23	37.0
Dry Auglaize	imp	14.0	10.0	2.5	7.4	1.87	0.25	44.5
Mean		**14.9**	**9.0**	**3.5**	**7.1**	**1.86**	**0.24**	**38.1**
SD		**3.4**	**2.6**	**4.1**	**1.5**	**0.38**	**0.09**	**12.8**
t-test, p values Ref/Imp		0.006	0.011	0.044	0.012	0.068	0.249	0.711

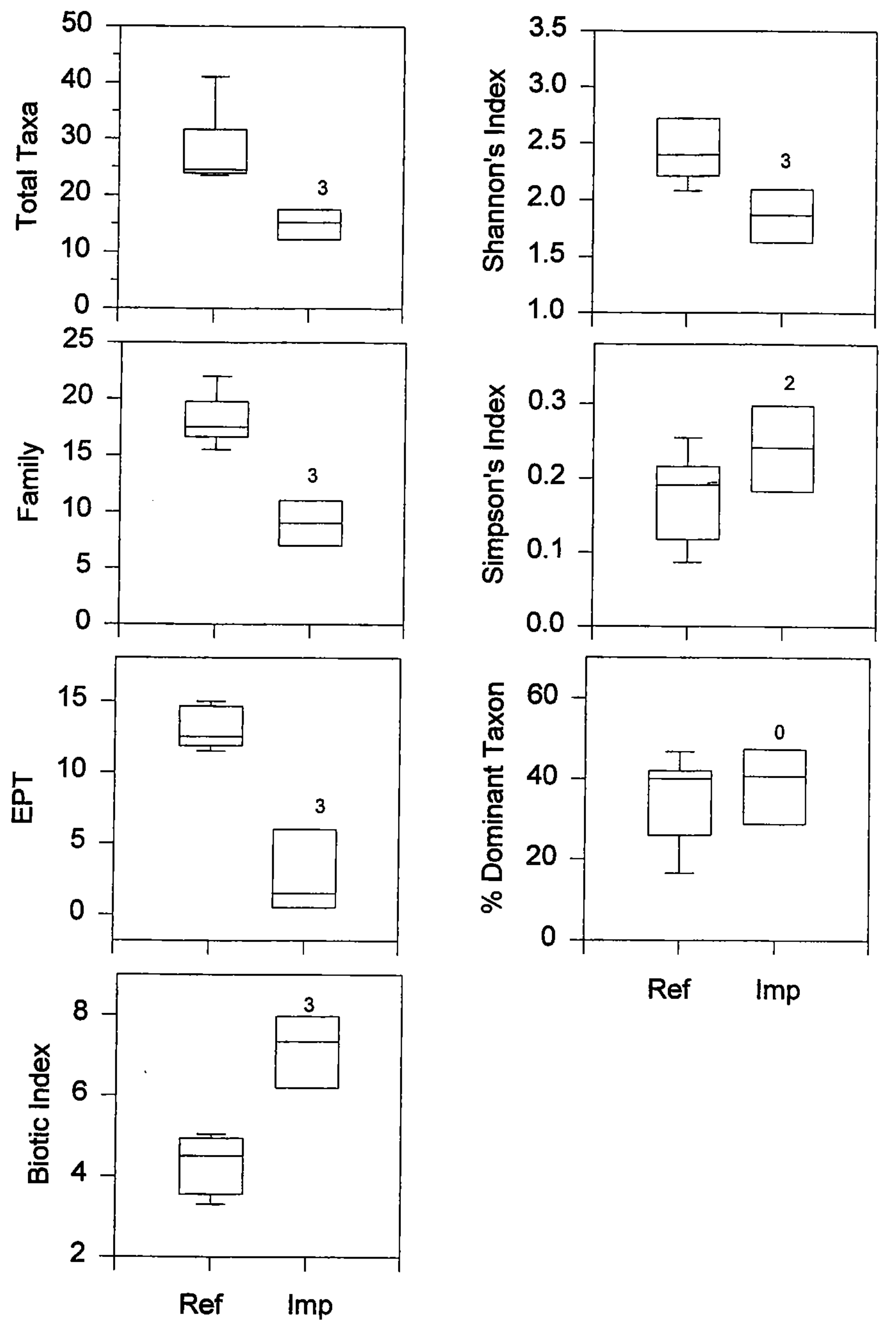


Fig. 13. Box and whisker plots comparing reference (REF) and impaired (IMP) streams using single habitat data, fall 1994. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

PART B. SENSITIVITY ANALYSIS SUMMER 1995

Introduction

The summer 1995 effort was a continuation of fall 1994 objectives to examine the ability of using benthic invertebrates to distinguish both water quality degradation and habitat degradation. Specifically the research questions were to: 1) evaluate the sensitivity of the seven metrics to both water quality degradation and habitat degradation; 2) evaluate the utility of "paired metrics"; 3) determine the utility of data collected from multihabitats vs. data collected from a single habitat.

Methods

A somewhat different experimental design was used for the summer 1995 effort. Instead of using a randomly selected group of reference streams, we selected pairs of streams from the same general locality with similar size and hydrologic regime. The major distinction was one of the pair was of reference quality, while the other was impaired, either because of organic enrichment or because of habitat degradation. All streams were from the Ozark ecoregion. Ten paired streams were selected to compare REF sites to HAB sites (Fig. 14, Table 18). One pair was later deleted (Brush and Dousinberry creeks) because a fish kill was discovered in the reference stream. Eight paired streams were used to compare reference to ORG streams (Fig. 14, Table 18). At each site all available habitats were sampled (Table 19), discharge measurements taken, water samples for nutrient analysis obtained, and habitat analysis completed (Table 20).

Several different analyses were conducted on the invertebrate data. Community structure was examined using DCA ordination so as to visualize relative similarities among communities. We then calculated metrics and examined for significant differences between stream types. Because streams were paired, a paired t-test was used to evaluate each metric. We compared metric similarities between paired streams as the percent similarity of the degraded stream metric value to the REF value calculated as

1 -{(REF value - Degraded site value) / REF value)} X 100.

We next examined the utility of "paired metrics" for this project. Finally, we examined correlations between metrics and environmental variables.

Results

Analyses Using Multihabitat (cs flow and nonflow)

Although five habitat types were sampled whenever they were encountered, only cs flow, nonflow, and rootmat were commonly found. For consistency among all sites, only cs flow and nonflow were used in the analysis. A comparison of community structure among all streams was done by using DCA. REF sites separated out quite well from HAB sites with a single overlap (Fig. 15). REF sites were well grouped together, while HAB sites showed two separate groupings. In the REF-ORG sites comparison there was also good separation (Fig. 15). Only one stream, (Dry Auglaize Creek) was interspersed. This analysis shows definite differences in community structure between REF and each type impairment.

Metric Similarity Between Paired Sites

We considered a deviation of >25% from the REF value for any metric to be

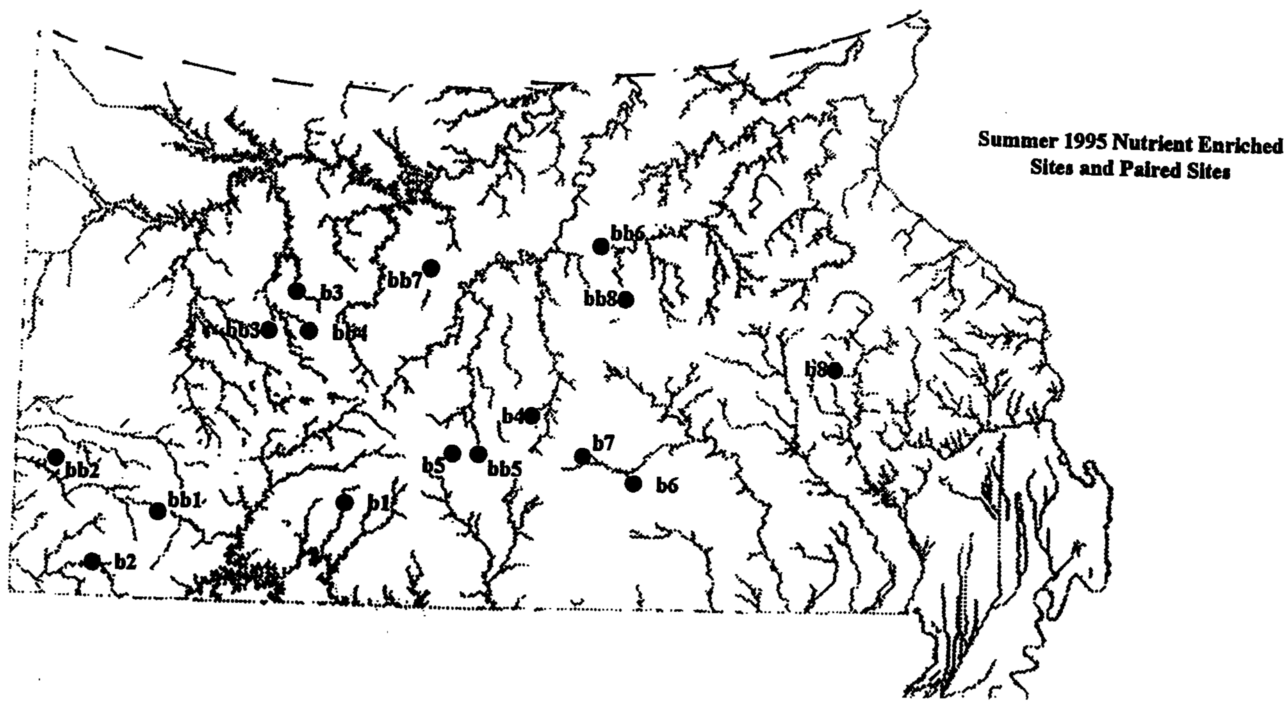


Fig. 14. Locations of the 1995 habitat impacted (a) and corresponding paired (aa) sites and the nutrient enriched (b) and corresponding organically enriched (bb) sites.

Table 18. Summer 1995 Sampling Locations

Stream #	Designated Stream Condition	Stream	Site Location	Comments
1a	Nutrient enriched	Clear Cr.	Barry Co.; Sec. 29; T26N; R28W.	at Monett STP
1b	Reference	Swan Cr.	Christian Co.; Sec. 15; T26N; R19W.	
2a	Habitat disturbed	Dry Auglaize Cr.	Camden Co.; N 1/2; Sec. 23; T38N; R15W.	
2b	Reference	Little Niangua R.	Hickory Co.; NW 1/4; Sec. 2; T37N; R20W.	at Mule Shoe WA
3a	Habitat disturbed	Greasy Cr.	Dallas Co.; Sec. 27; T33N; R20W.	
3b	Reference	Starks Cr.	Hickory Co.; S 1/2; Sec. 20; T38N; R20W.	
4a	Habitat disturbed	Dousinberry Cr.	Dallas Co.; Sec. 12; T33N; R19W.	
4b	Reference	Brush Cr.	Laclede Co.; SE 1/4; Sec. 27; T33M; R16W.	
5a	Habitat disturbed	Cole Camp Cr.	Benton Co.; Sec. 10; T41N; R21W.	
5b	Reference	Deer Cr.	Benton Co.; border Secs. 30 & 31; T40N; R20W.	
6a	Nutrient enriched	Turkey Cr.	Jasper Co.; border Secs. 29 & 30; T28N; R33W.	
6b	Reference	Big Sugar Cr.	McDonald Co.; S 1/2; Sec.26; T30N; R22W	private access
7a	Habitat disturbed	Clark Cr.	Wright Co.; Sec. 36; T30N; R14W	at Hway. 38
7b	Reference	Woods Fk.	Wright Co.; SW 1/4; Sec. 32; T30N; R15W.	
8a	Nutrient enriched	Piper Cr.	Polk Co.; W 1/2; Sec. 31: T34N; R22W.	
8b	Reference	Lindley Cr.	Polk Co.; E 1/2; Sec. 4: T34N; R21W.	
9a	Nutrient enriched	Hominey Cr.	Polk Co.; border Secs. 26 & 35; T34N; R22W.	at Hway. 22
9b	Reference	W. Piney Cr.	Texas Co.; NW 1/4 Sec. 20; T30N; R10W.	
10a	Habitat disturbed	Hutchins Cr.	Dent Co.; Sec. 10; T34N; R4W.	
10b	Reference	E. Fk. Huzzah Cr.	Dent Co.; E 1/2; NE 1/4; NE 1/4; Sec. 1; T34N; R3W.	private access
11a	Nutrient enriched	Little Dry Br.	Phelps Co.; border Secs. 8 & 17; T37N; R7W.	
11b	Reference	Shawnee Cr.	Shannon Co.; Sec. 30; T29N: R3W.	
12a	Nutrient enriched	Dry Auglaize Cr.	Laclede Co.; Sec. 30; T35N; R15W.	
12b	Reference	N. Prong Jacks Fk.	Texas Co.; Sec. 4; T28N; R8W.	
13a	Nutrient enriched	Spring Br.	Dent Co.; Sec. 32; T35N; R6W.	
13b	Reference	Marble Cr.	Madison Co.; N 1/2; Sec. 19; T32N; R5E.	
14a	Habitat disturbed	Crooked Cr.	Crawford Co.; Sec. 8; T36; R4W.	
14b	Reference	Crane Pond Cr.	Iron Co.; SE 1/4; Sec. 10; T31N; R4E.	
15a	Habitat disturbed	Big Cr.	Iron Co.; Sec. 8; T30N; R4E.	
15b	Reference	Huzzah CR.	Crawford Co.; S 1/2; Sec. 29; T36N; R2W.	
16a	Habitat disturbed	Indian Cr.	Washington Co.; S 1/2; Sec. 13; T40N; R1W.	
16b	Reference	S. Fk. Little Meramec R.	Franklin Co.; SEc. 7; T41N; R2E.	
17a	Habitat disturbed	Little Tavern Cr.	Miller Co.; border Secs. 25 & 26; T41N; R12W.	
17b	Reference	Maries R.	Maries Co.; border Secs. 7 & 8; T39N; R10W.	
18a	Nutrient enriched	E. Fk. Whetstone Cr.	Wright Co.; NE 1/4; NE 1/4; Sec. 5; T28N; R13W.	at Mt. Grove STP
18b	Reference	Whetstone Cr.	Wright Co.; center Sec. 21; T29N; R13W.	

Table 19. Habitat types sampled for each paired streams, summer 1995

Reference streams	No.	csfl	nonfl	root	snag	veg	Hab. degraded streams	No.	csfl	nonfl	root
				Comparison of reference to habitat degraded streams							
Ltl. Niangua	a1	X	X	X		X	Dry Aug.(C)	aa1	X	X	
Starks	a2	X	X	X		X	Greasy	aa2	X	X	
Brush	a3	X	X				Dusinberry	aa3	X	X	
Deer	a4	X	X	X		X	Cole camp	aa4	X	X	
Wooks Fk	a5	X	X	X			Clark	aa5	X	X	
E Fk Huzz	a6	X	X	X			Hutchins	aa6	X	X	X
Crand pond	a7	X	X	X			Crooked	aa7	X	X	X
Huzzah	a8	X	X	X			Big Cr (Iron)	aa8	X	X	
Meramec	a9	X	X	X			Indian	aa9	X	X	
Maries	a10	X	X	X			Ltl Tavern	aa10	X	X	
				Comparison of reference to organically enriched streams							
Reference streams							Org. enriched streams				
Swan	b1	X	X	X			Clear	bb1	X	X	X
Big Sugar	b2	X	X		X		Turkey	bb2	X	X	X
Lindley	b3	X	X			X	Piper	bb3	X	X	
W Piney	b4	X	X			X	Hominy	bb4	X	X	
Whetstone	b5	X	X			X	E FK Whets	bb5	X	X	X
Shawnee	b6	X	X	X			Ltl Lindley	bb6	X	X	X
N Jacks	b7	X	X			X	Dry Aug. (L)	bb7	X	X	X
Marble	b8	X	X	X			Spring	bb8	X	X	X

Table 20. Dischage, habitat score, total phosphorus and total nitrogen, summer 1995

Streams		Discharge (CFS)		Habitat score		Tot.-P (ug/L)		Tot.-N (mg/L)	
		Ref	Hab	Ref	Hab	Ref	Hab	Ref	Hab
Reference	**Hab.degra.**								
Ltl. Niangua	Dry Aug.(C)	36.01	6.68	110	77	22	52	0.16	0.22
Starks	Greasy	2.36	12.26	132	89	22	54	0.22	0.45
Brush	Dusinberry	2.20	8.57	116	93	16	29	0.46	0.25
Deer	Cole camp	6.68	6.79	113	81	15	42	0.08	0.35
Wooks Fk	Clark	1.58	3.64	105	91	26	140	0.41	0.98
E Fk Huzz	Hutchins	11.39	3.17	138	79	6	6	0.17	0.16
Crand pond	Crooked	1.89	6.55	109	77	6	14	0.14	0.22
Huzzah	Big Cr (Iron)	80.75	26.77	136	92	6	6	0.22	0.09
Meramec	Indian	2.07	27.53	117	82	27	4	0.63	0.10
Maries	Ltl Tavern	2.17	19.60	114	67	17	28	0.20	0.26
Reference	**Org. enrich.**								
Swan	Clear	2.50	6.50	118	117	5	12700	0.26	24.76
Big Sugar	Turkey	68.84	29.49	118	116	19	1350	1.02	3.76
Lindley	Piper	5.10	3.62	120	125	67	2645	0.41	3.91
W Piney	Hominy	17.88	3.10	131	128	14	55	0.22	0.54
Whetstone	E FK Whets	4.02	0.59	116	88	28	1504	0.48	2.10
Shawnee	Ltl Lindley	11.61	4.72	118	106	8	1352	0.26	10.10
N Jacks	Dry Aug. (L)	2.56	1.36	127	87	5	2875	0.21	7.47
Marble	Spring	8.89	14.66	129	137	12	55	0.25	1.11

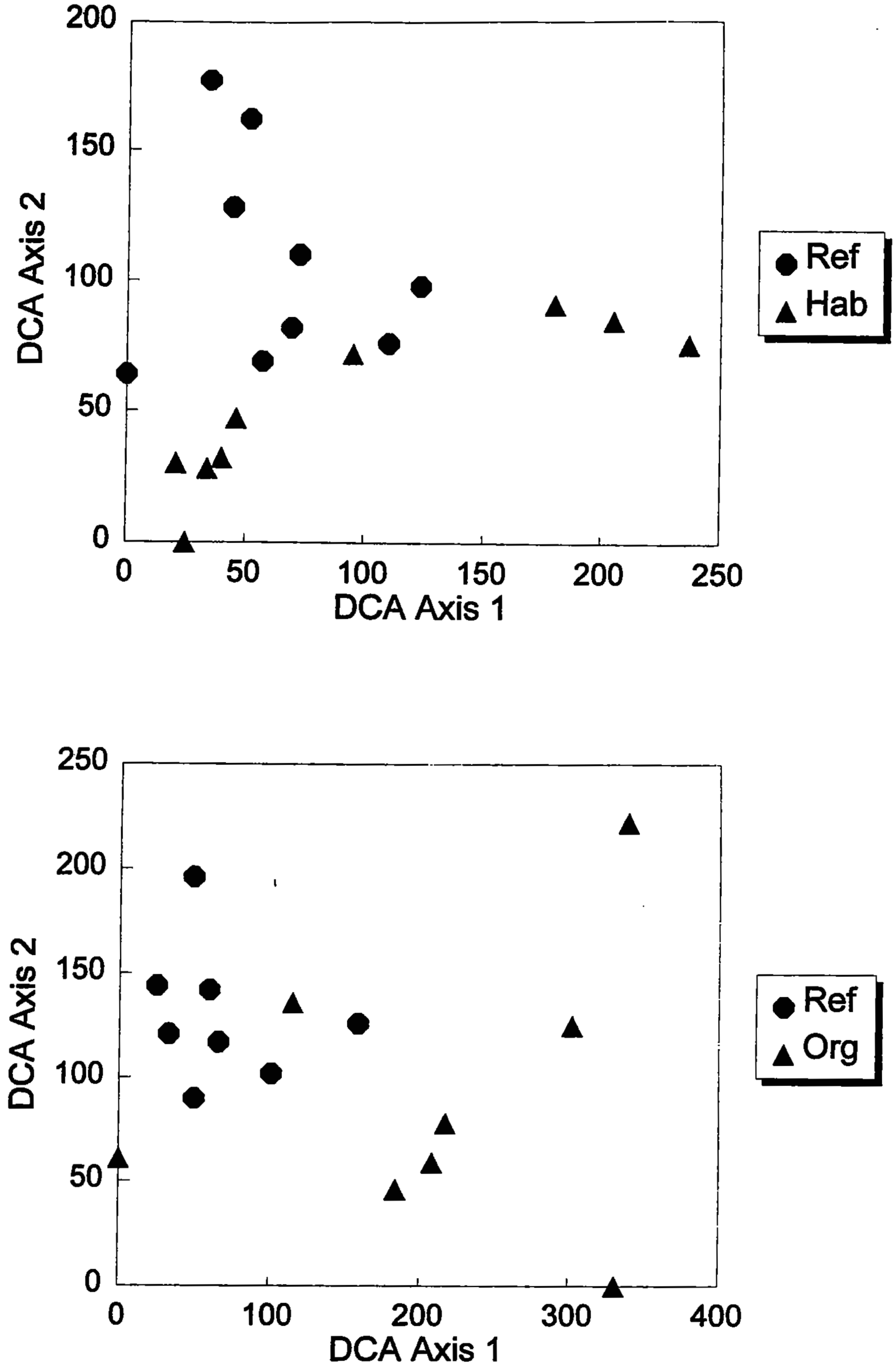


Fig. 15. Ordination of habitat degraded sites and corresponding reference sites, and organically enriched sites and corresponding reference sites, multihabitat, 1995.

impairment. For the REF-HAB site comparisons, most sites were not distinguishable as being impaired by most of the metrics (Table 21). Only Simpson's diversity index and % Dominant taxon —two metrics generally shown to be insensitive to degradation—showed good ability to distinguish degraded sites. However, for REF-ORG site comparisons, all metrics showed good ability to distinguish organically enriched streams.

Mean Metric Differences

Each metric was statistically analyzed to determine differences between REF streams and HAB streams (Table 22). The only significant difference at $P < 0.05$ was for the Family metric, although others had low p values—e.g., a P of 0.18 for Total taxa and $P = 0.096$ for Shannon's diversity index. However, for the REF-ORG comparison, every metric had significant differences (Table 22). These results are consistent with the trend of many metrics having a good ability to detect organic pollution, but a lesser sensitivity to habitat degradation.

Box and Whisker Plots

There was very little discrimination between REF and HAB sites. Only the Shannon's diversity index and Simpson's diversity index showed any sensitivity (Fig. 16). ORG sites were easily distinguished from REF streams (Fig. 17). All metrics showed some level of sensitivity, with Total taxa, EPT, BI, and Shannon's diversity index showing maximum sensitivity.

The majority of situations deemed to be moderately or very sensitive by the Box and Whisker plots were also statistically significantly different. The Box and Whisker analysis is more liberal in designating some of the HAB sites as different than are the statistics.

Associations Between Metric Scores and Environmental Variables

No significant correlation was found between stream discharge and any metric ($P > 0.05$; Table 23). For the REF-HAB comparisons there was a significant correlation between metric values and habitat scores only for the Family and Shannon's diversity index metrics ($P <$ 0.05;), the other five metrics were not significantly different. No metric from a HAB stream was significantly related to TN or TP.

In the REF-ORG comparisons there were significant correlations between TN and TP for all seven metric $P < 0.05$; Table 23), except that Total taxa and % Dominant taxon were not significantly correlated with TN. However, EPT and BI were significantly correlated with habitat scores.

Analyses Using a Single Habitat (cs flow)

Community Structure

Separation of REF sites from HAB sites was fair (Fig. 18). REF sites tended to be grouped together while HAB were more dispersed. Separation was not quite as good as with multihabitat data. For REF–ORG sites ordination we see a good separation between the two classes of streams (Fig. 18). REF sites grouped more tightly, implying a basic similarity of community structure, than did the ORG, implying a more diverse group of sites.

Metric Similarity

For REF-HAB sites every pair of streams but one had one or more metric indicating impairment (Table 24); however, only Simpson's diversity index and % Dominant taxon were able to show consistent impairment. Better distinctions

Table 21. The percentage similarity of each metric value of a habitat impaired or organically enriched site compared to its paired reference site, multihabitat, summer 1995. Similarity <75 are marked with asterisk.

Ref.	Hab. deg.	Taxa	Family	Ref./Hab. EPT	Biotic Ind	Shannon	Simpson	Dominant
Ltl. Niangua	Dry Aug.(C)	83.3	81.0	78.9	95.8	98.6	109.5	115.8
Starks	Greasy	78.0	88.9	80.0	101.2	88.6	77.1	107.0
Deer	Cole camp	118.2	109.5	105.9	106.7	80.3	45.0 *	57.1 *
Wooks Fk	Clark	93.2	83.3	86.4	105.0	110.4	146.8	145.6
E Fk Huzz	Hutchins	100.0	92.9	109.1	91.7	90.9	61.7 *	56.1 *
Crand pond	Crooked	87.2	104.3	125.0	106.6	88.9	55.8 *	47.7 *
Huzzah	Big Cr (Iron)	89.1	85.7	66.7 *	96.0	101.4	119.4	118.8
Meramec	Indian	114.0	92.9	143.8	106.5	100.1	93.8	92.9
Maries	Ltl Tavern	83.3	73.3 *	94.1	103.9	89.9	65.3 *	74.6 *

Ref.	Org. enriched	Taxa	Family	Ref./Org. EPT	Biotic Ind	Shannon	Simpson	Dominant
Swan	Clear	49.0 *	48.0 *	5.0 *	60.7 *	60.2 *	48.4 *	76.9
Big Sugar	Turkey	51.1 *	68.2 *	57.1 *	86.1	59.2 *	36.0 *	63.1 *
Lindley	Piper	156.7	115.8	125.0	86.6	115.5	155.8	154.9
W Piney	Hominy	67.2 *	69.7 *	50.0 *	74.0 *	73.4 *	32.5 *	41.9 *
Whetstone	E FK Whets	73.8 *	72.7 *	5.6 *	59.1 *	62.3 *	27.3 *	37.4 *
Shawnee	Ltl Lindley	49.2 *	43.8 *	16.7 *	72.4 *	69.2 *	44.3 *	61.4 *
N Jacks	Dry Aug. (L)	82.0	96.4	50.0 *	85.6	98.1	118.8	150.6
Marble	Spring	83.7	91.3	47.8 *	108.7	91.4	84.1	96.7

Table 22. Metrics for all paried streams using multihabitat data of summer 1995.

	Reference / Habitat Degraded Streams						
	Taxa	Family	EPT	Biotic Ind	Shannon	Simpson	Dominant
Reference streams							
Ltl. Niangua	48	21	19	5.5	3.18	0.07	19.1
Starks	59	27	20	6.0	3.19	0.07	19.8
Deer	44	21	17	6.0	3.24	0.06	15.3
Wooks Fk	59	30	22	5.9	3.01	0.09	21.7
E Fk Huzz	51	28	22	4.7	3.23	0.06	12.0
Crand pond	47	23	16	5.0	3.00	0.08	14.8
Huzzah	55	28	24	5.0	2.91	0.12	31.0
Meramec	50	28	16	6.3	2.85	0.12	27.6
Maries	54	30	17	5.9	2.68	0.14	31.2
Mean	**51.9**	**26.2**	**19.2**	**5.6**	**3.03**	**0.09**	**21.4**
SD	**5.3**	**3.6**	**2.9**	**0.6**	**0.19**	**0.03**	**7.1**
Habitat degraded streams							
Dry Aug.(C)	40	17	15	5.7	3.13	0.06	16.5
Greasy	46	24	16	5.9	2.83	0.10	18.5
Cole camp	52	23	18	5.6	2.60	0.13	26.8
Clark	55	25	19	5.6	3.32	0.06	14.9
Hutchins	51	26	24	5.1	2.94	0.09	21.4
Crooked	41	24	20	4.7	2.67	0.14	31.0
Big Cr (Iron)	49	24	16	5.2	2.95	0.10	26.1
Indian	57	26	23	5.9	2.86	0.13	29.7
Ltl Tavern	45	22	16	5.7	2.41	0.21	41.8
Mean	**48.4**	**23.4**	**18.6**	**5.5**	**2.85**	**0.11**	**25.2**
SD	**5.9**	**2.7**	**3.2**	**0.4**	**0.28**	**0.05**	**8.5**
Paired t-test	**0.186**	**0.025**	**0.678**	**0.4**	**0.10**	**0.10**	**0.206**

	Reference / Organically enriched Streams						
	Taxa	Family	EPT	Biotic Ind	Shannon	Simpson	Dominant
Reference streams							
Swan	49	25	20	5.1	2.80	0.14	33.3
Big Sugar	45	22	14	6.2	3.02	0.09	21.9
Lindley	30	19	12	6.5	2.57	0.12	26.8
W Piney	61	33	22	5.7	3.37	0.05	12.5
Whetstone	42	22	18	5.2	3.08	0.06	14.3
Shawnee	59	32	24	5.7	3.08	0.09	25.5
N Jacks	50	28	22	5.7	2.98	0.10	24.7
Marble	49	23	23	5.4	2.98	0.09	23.7
Mean	**48.1**	**25.5**	**19.4**	**5.7**	**2.98**	**0.09**	**22.8**
SD	**9.7**	**5.0**	**4.4**	**0.5**	**0.23**	**0.03**	**6.7**
Organicall enriched streams							
Clear	24	12	1	8.4	1.69	0.28	43.3
Turtey	23	15	8	7.2	1.79	0.24	34.7
Piper	47	22	15	7.5	2.96	0.08	17.3
Hominy	41	23	11	7.7	2.47	0.15	29.8
E FK Whets	31	16	1	8.7	1.92	0.23	38.2
Ltl Lindley	29	14	4	7.8	2.13	0.21	41.5
Dry Aug. (L)	41	27	11	6.7	2.92	0.08	16.4
Spring	41	21	11	4.9	2.72	0.11	24.5
Mean	**34.6**	**18.8**	**7.8**	**7.4**	**2.33**	**0.17**	**30.7**
SD	**9.0**	**5.2**	**5.2**	**1.2**	**0.51**	**0.08**	**10.5**
Paired t-test	**0.035**	**0.027**	**0.003**	**0.009**	**0.018**	**0.027**	**0.112**

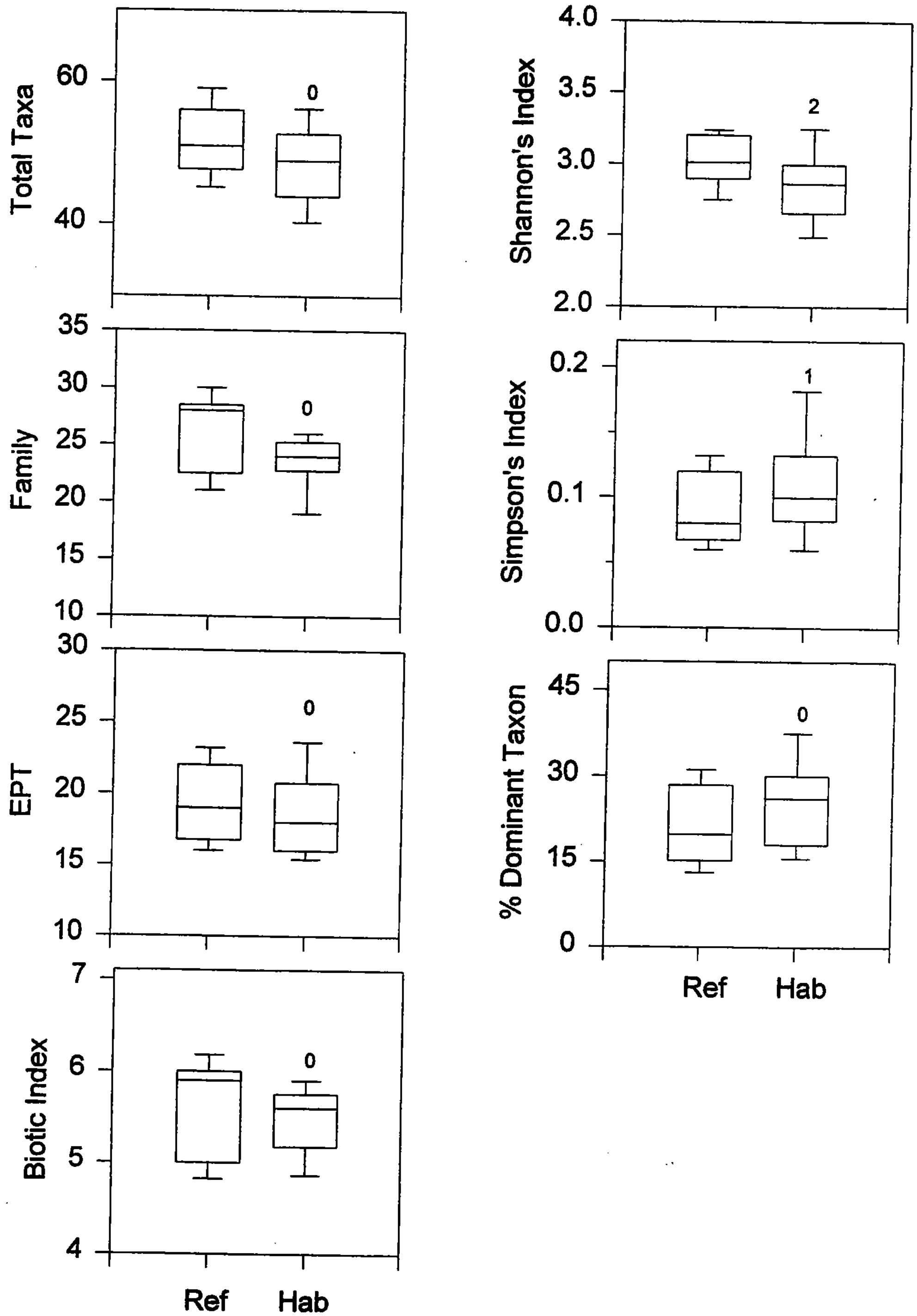


Fig. 16. Discriminatory power analysis for metrics examining reference vs. habitat degraded sites, multihabitat 1995. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

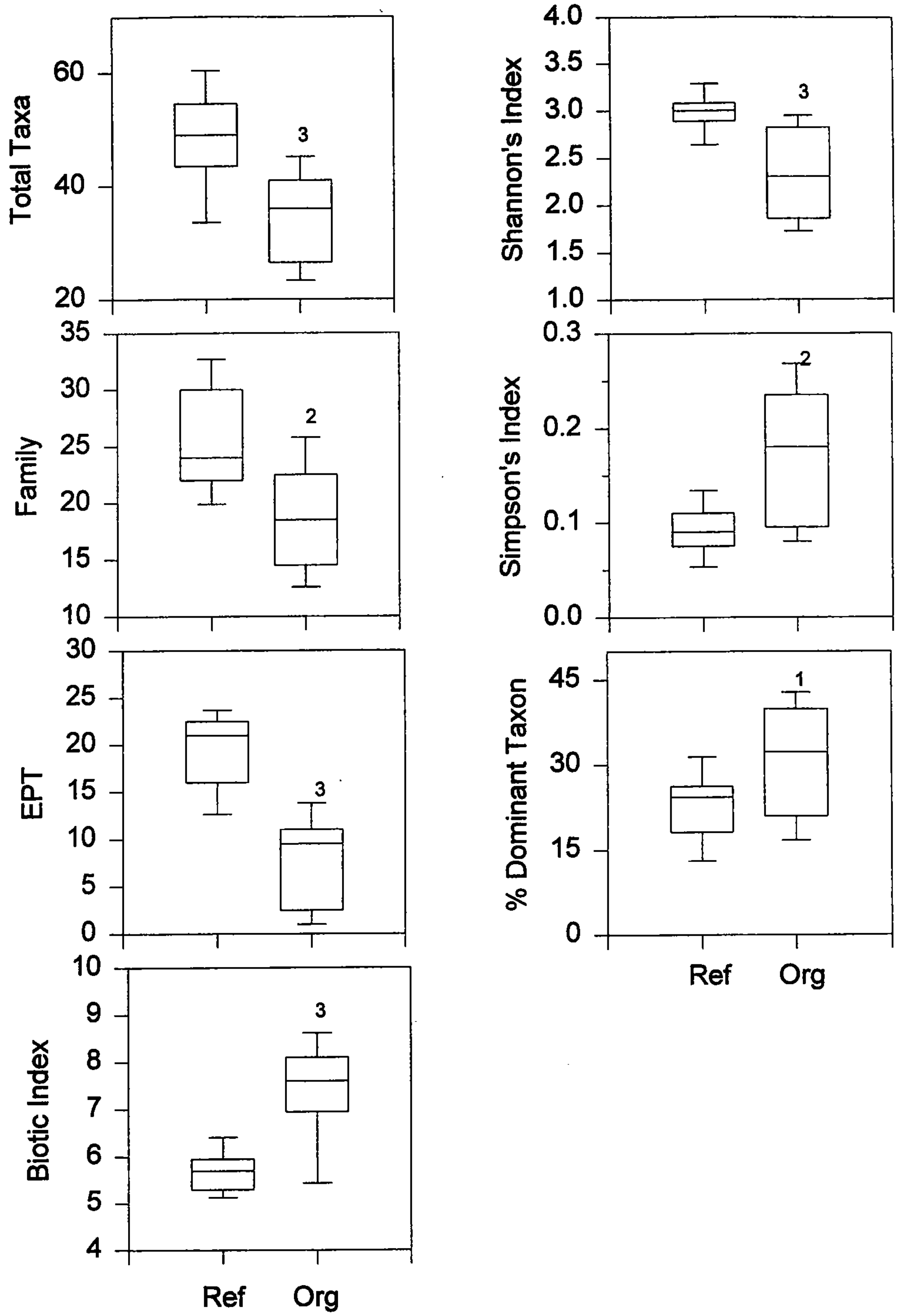


Fig. 17. Discriminatory power analysis for metrics examining reference vs. organically enriched sites, multihabitat 1995. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

Table 23. Correlation coefficient (r) between metric values and environmental variables, Ozark, summer 1995. Significant correlations ($p<0.05$) are in bold.

		cs flow+nonflow habitats				cs flow habitat			
Metrics	N	Discharge	Hab. Score	Tot.-N	Tot.-P	Discharge	Hab. Score	Tot.-N	Tot.-P
Reference/Habitat Degraded Streams									
Taxa	18	0.119	0.425	0.036	0.219	0.007	0.378	0.247	0.296
Family	18	0.030	**0.509**	0.187	0.185	0.046	0.216	**0.641**	**0.696**
EPT	18	0.365	0.246	0.251	0.126	0.195	0.285	0.295	0.255
Biotic Index	18	0.298	0.043	0.321	0.344	0.368	0.289	**0.538**	**0.553**
Shannon	18	0.060	**0.487**	0.252	0.115	0.070	**0.594**	0.291	0.183
Simpson	18	0.193	0.416	0.211	0.063	0.015	**0.664**	0.264	0.177
Dominant	18	0.322	0.335	0.264	0.113	0.104	**0.615**	0.285	0.227
Reference/Organically Enriched Streams									
Taxa	16	0.052	0.397	0.486	**0.563**	0.080	0.205	0.423	0.468
Family	16	0.007	0.268	**0.502**	**0.573**	0.118	0.254	**0.589**	**0.598**
EPT	16	0.047	**0.511**	**0.561**	**0.636**	0.040	0.427	**0.567**	**0.603**
Biotic Index	16	0.132	**0.513**	**0.550**	**0.578**	0.317	**0.526**	**0.579**	**0.614**
Shannon	16	0.117	0.367	**0.561**	**0.611**	0.090	0.191	0.344	0.396
Simpson	16	0.099	0.342	**0.591**	**0.642**	0.004	0.166	0.162	0.217
Dominant	16	0.135	0.266	0.451	**0.536**	0.060	0.173	0.010	0.055

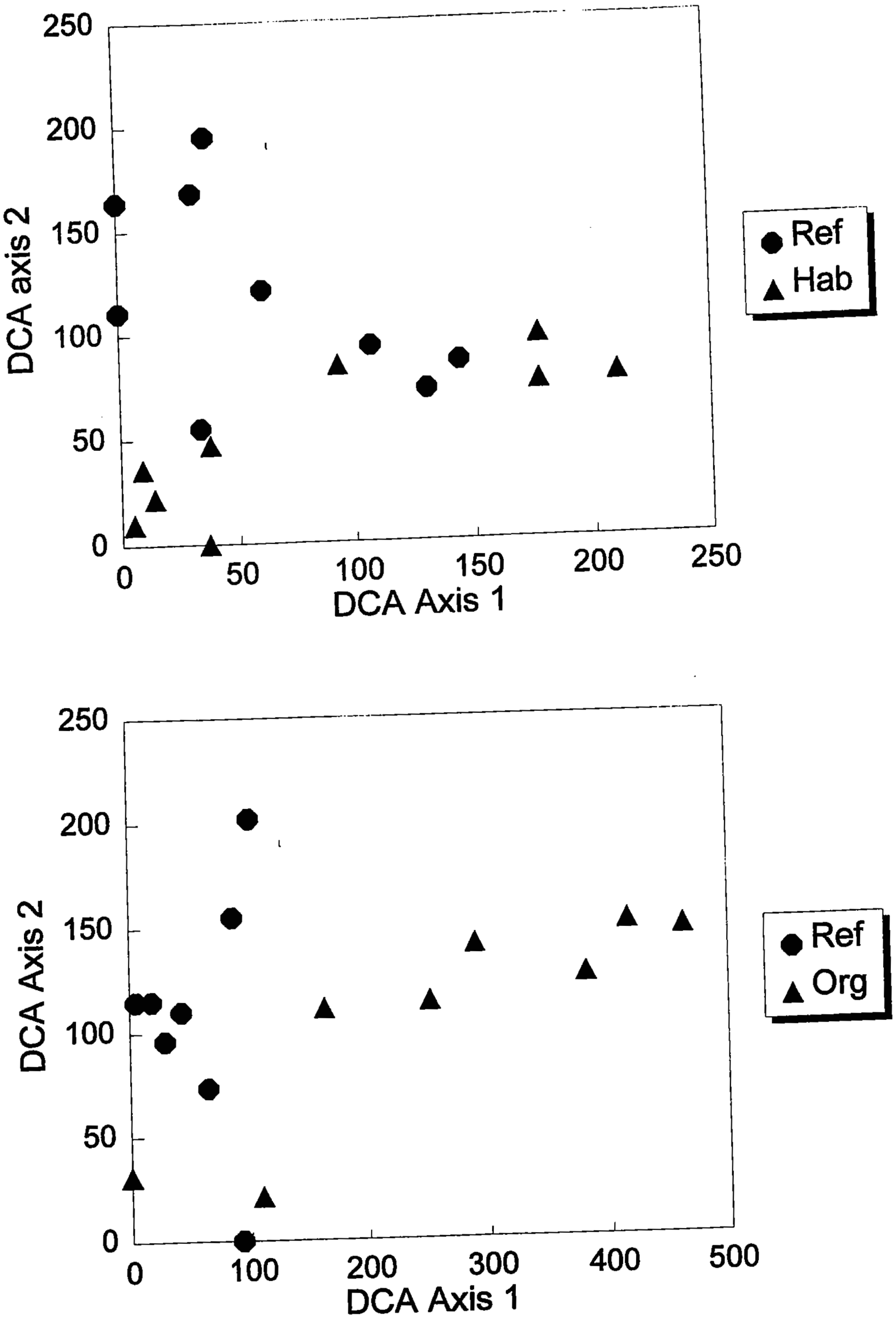


Fig. 18. Ordination of habitat degraded sites and corresponding reference sites, and organically enriched sites and corresponding reference sites, single habitat, (cs flow) 1995.

Table 24. The percentage similarity of each metric value of a habitat impaired or organically enriched site compared to its paired reference site using cs flow habitat data of summer 1995. Similarity <75 are marked with asterisk.

Ref.	Hab. deg.	Taxa	Family	Ref./Hab. EPT	Biotic Ind	Shannon	Simpson	Dominant
Ltl. Niangua	Dry Aug.(C)	84.8	82.4	86.7	74.5 *	95.9	85.6	72.6 *
Starks	Greasy	81.0	75.0	66.7 *	94.5	80.9	45.6 *	47.9 *
Deer	Cole camp	120.0	94.1	133.3	100.4	80.0	41.0 *	37.9 *
Wooks Fk	Clark	100.0	194.7	77.8	89.3	108.5	145.3	163.9
E Fk Huzz	Hutchins	94.1	95.0	100.0	84.0	92.1	48.6 *	78.7
Crand pond	Crooked	127.3	142.9	123.1	95.5	94.7	55.4 *	44.4 *
Huzzah	Big Cr (Iron)	79.4	81.8	72.2 *	94.7	80.9	46.2 *	53.1 *
Meramec	Indian	109.7	90.5	172.7	121.0	103.7	91.0	72.7 *
Maries	Ltl Tavern	74.3 *	66.7 *	64.7 *	96.9	78.6	44.9 *	55.5 *

Ref.	Org. enriched	Taxa	Family	Ref./Org. EPT	Biotic Ind	Shannon	Simpson	Dominant
Swan	Clear	36.4 *	15.8 *	0.0 *	65.2 *	70.5 *	76.6	128.1
Big Sugar	Turkey	32.3 *	25.0 *	0.0 *	67.3 *	37.6 *	19.0 *	36.6 *
Lindley	Piper	131.8	86.7	41.7 *	78.5	123.5	216.3	269.1
W Piney	Hominy	50.0 *	57.7 *	55.6 *	84.7	74.4 *	38.0 *	35.1 *
Whetstone	E FK Whets	57.1 *	41.2 *	0.0 *	53.3 *	63.3 *	31.2 *	34.0 *
Shawnee	Ltl Lindley	50.0 *	45.8 *	23.5 *	58.4 *	63.6 *	35.2 *	44.1 *
N Jacks	Dry Aug. (L)	93.5	90.9	45.0 *	87.6	105.1	132.6	134.4
Marble	Spring	70.8 *	87.5	53.3 *	104.0	83.8	62.5 *	50.3 *

were shown for the REF-ORG site comparisons, where every metric indicated at least half the streams were affected (Table 24). The EPT metric distinguished every pair of streams. These results were similar to or, in some cases, better than the multihabitat data.

Mean Metric Differences

For REF-HAB site comparisons, only the two diversity indices and the % Dominant taxon showed statistically significant differences (Table 25). However, this was one more metric than was significant when using multihabitat data. For the REF-ORG comparisons, Total taxa, Family, EPT, BI, and Shannon's diversity index showed significant differences which was the same result as when using multihabitat data.

Box and Whisker Plots

The discrimination between REF and HAB sites was good (Fig. 19). Every metric showed some degree of sensitivity; and Shannon's diversity Index, Simpson's diversity Index, and % Dominant taxon showed maximum sensitivity. ORG sites were easily distinguished from reference streams (Fig. 20). All metrics showed the highest level of sensitivity (3) except % Dominant taxon which showed a value of 2.

Greater overall sensitivity was observed using a single habitat analysis than using multihabitat data.

Associations Between Metric Scores and Environmental Variables

There were no significant correlations between discharge and any metric ($p > 0.05$; Table 23). Correlations between habitat scores and metrics were significant for the two diversity indices and % Dominant taxon ($P < 0.05$; Table 25). Only Family and BI were significantly correlated with TN and TP. These results show that the two diversity indices and % Dominant taxon were sensitive to habitat degradation and were more consistent with metric comparisons (see Table 11). These metrics were not correlated with nutrient levels. Single habitat results are clearer than those with multihabitat data.

There were significant correlations between metric scores and TN and TP for Family, EPT, and BI ($P < 0.05$; Table 23). However, the BI was also significantly related to habitat scores. These results were not as clear as those from multihabitat data.

Part B Conclusion

Results of our metric sensitivity analyses for 1995 sampled sites indicate several points: 1) organically affected streams are readily discernible from REF streams by five metrics—Total taxa, Family, EPT, BI, and Shannon's diversity index; 2) HAB sites were not as readily detected by most metrics (while there was more difficulty in detecting HAB streams, the two diversity indices and % Dominant taxon were most sensitive); 3) overall, with many comparisons, there was nearly equal sensitivity using multi- or single habitat analysis, or single habitat was better; in no case was multihabitat superior. Box and whisker plot analyses appear consistent with other analyses, are readily interpretable, and are biologically justifiable.

Table 25. Metrics for all paired streams using cs flow habitat data of summer 1995.

	Reference / Habitat Degraded Streams						
	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson	Dominant
Reference streams							
Ltl. Niangua	33	17	15	4.0	2.92	0.08	15.9
Starks	42	24	18	5.1	3.05	0.07	13.4
Deer	30	17	12	5.5	3.02	0.06	10.7
Wooks Fk	36	19	18	4.9	2.91	0.08	17.7
E Fk Huzz	34	20	18	3.7	2.87	0.09	19.9
Crand pond	22	14	13	4.2	2.57	0.10	17.2
Huzzah	34	22	18	4.3	2.95	0.08	20.0
Meramec	31	21	11	5.9	2.49	0.14	25.8
Maries	35	24	17	5.1	2.75	0.11	25.8
Mean	**33.0**	**19.8**	**15.6**	**4.7**	**2.84**	**0.09**	**18.5**
SD	**5.1**	**3.2**	**2.7**	**0.7**	**0.19**	**0.02**	**4.8**
Habitat degraded streams							
Dry Aug.(C)	28	14	13	5.3	2.80	0.09	21.9
Greasy	34	18	12	5.4	2.47	0.15	28.0
Cole camp	36	16	16	5.5	2.42	0.14	28.2
Clark	36	18	14	5.5	3.16	0.05	10.8
Hutchins	32	19	18	4.4	2.64	0.18	25.3
Crooked	28	20	16	4.4	2.44	0.18	38.7
Big Cr (Iron)	27	18	13	4.6	2.39	0.17	37.7
Indian	34	19	19	4.9	2.58	0.16	35.5
Ltl Tavern	26	16	11	5.3	2.16	0.24	46.5
Mean	**31.2**	**17.6**	**14.7**	**5.0**	**2.56**	**0.15**	**30.3**
SD	**3.3**	**1.8**	**2.6**	**0.4**	**0.27**	**0.05**	**10.0**
Paired t-test	**0.390**	**0.128**	**0.607**	**0.224**	**0.034**	**0.006**	**0.005**

	Reference / Organically enriched Streams						
	Taxa	Family	EPT	Biotic Ind	Shannon	Simpson	Dominant
Reference streams							
Swan	33	19	18	4.9	2.56	0.15	33.7
Big Sugar	31	20	13	4.4	2.89	0.08	19.6
Lindley	22	15	12	5.5	1.99	0.27	48.7
W Piney	46	26	18	4.9	3.18	0.06	12.1
Whetstone	28	17	14	4.3	2.87	0.07	13.7
Shawnee	40	24	17	4.3	2.94	0.08	16.7
N Jacks	31	22	20	5.1	2.65	0.11	24.6
Marble	24	16	15	4.6	2.53	0.11	16.2
Mean	**31.9**	**19.9**	**15.9**	**4.8**	**2.70**	**0.12**	**23.2**
SD	**7.4**	**3.7**	**2.6**	**0.4**	**0.34**	**0.06**	**11.6**
Organically enriched streams							
Clear	12	3	0	7.6	1.80	0.20	26.3
Turkey	10	5	0	6.5	1.09	0.43	53.6
Piper	29	13	5	7.1	2.46	0.12	18.1
Hominy	23	15	10	5.8	2.37	0.16	34.5
E FK Whets	16	7	0	8.1	1.82	0.23	40.3
Ltl Lindley	20	11	4	7.3	1.87	0.23	37.9
Dry Aug. (L)	29	20	9	5.8	2.78	0.09	18.3
Spring	17	14	8	4.4	2.12	0.17	32.2
Mean	**19.5**	**11.0**	**4.5**	**6.6**	**2.04**	**0.20**	**32.7**
SD	**6.7**	**5.3**	**3.9**	**1.1**	**0.48**	**0.10**	**11.1**
Paired t-test	**0.015**	**0.004**	**0.000**	**0.006**	**0.035**	**0.133**	**0.262**

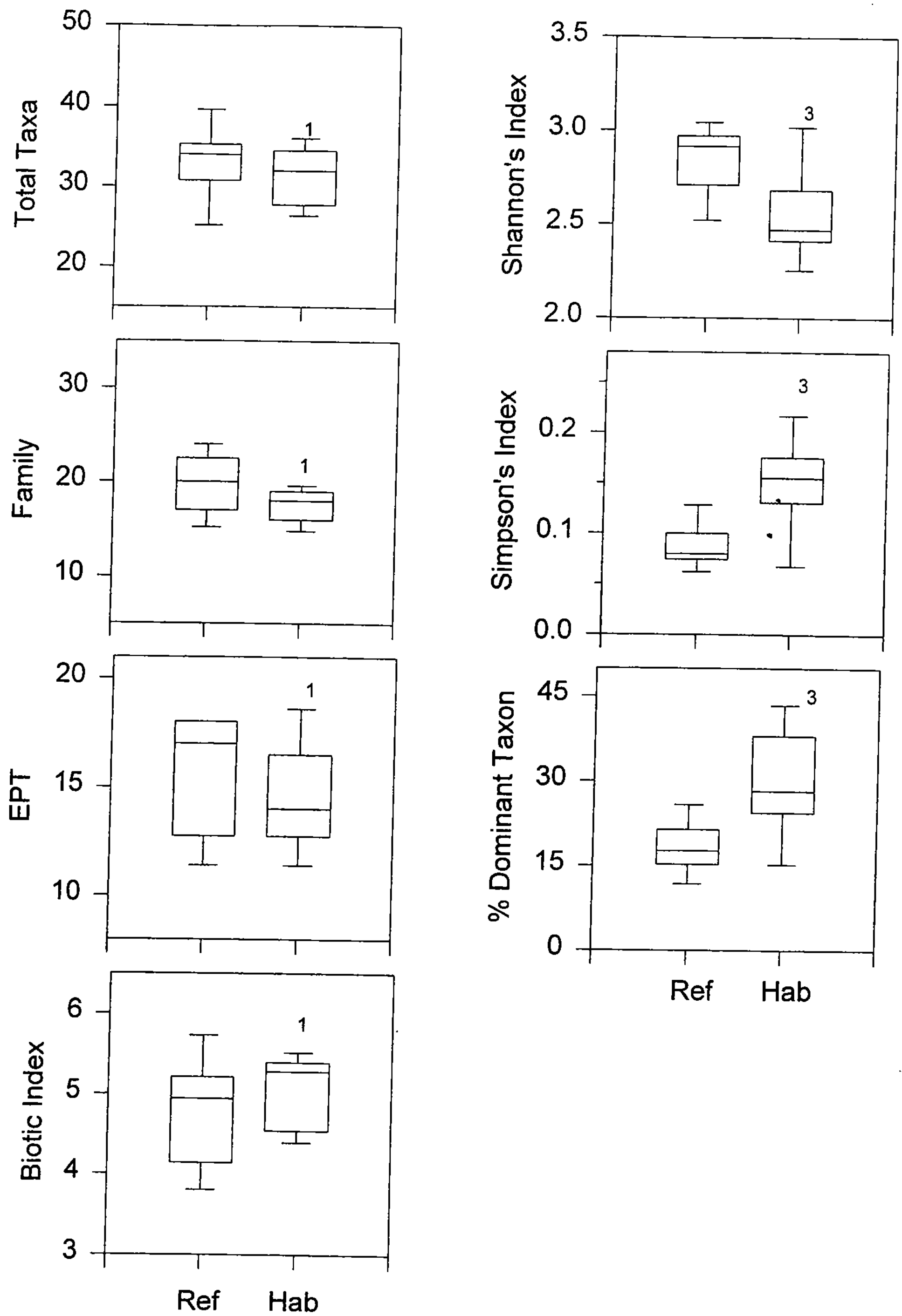


Fig. 19. Discriminatory power analysis for metrics examining reference vs. habitat degraded sites, single habitat (cs flow) 1995. Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

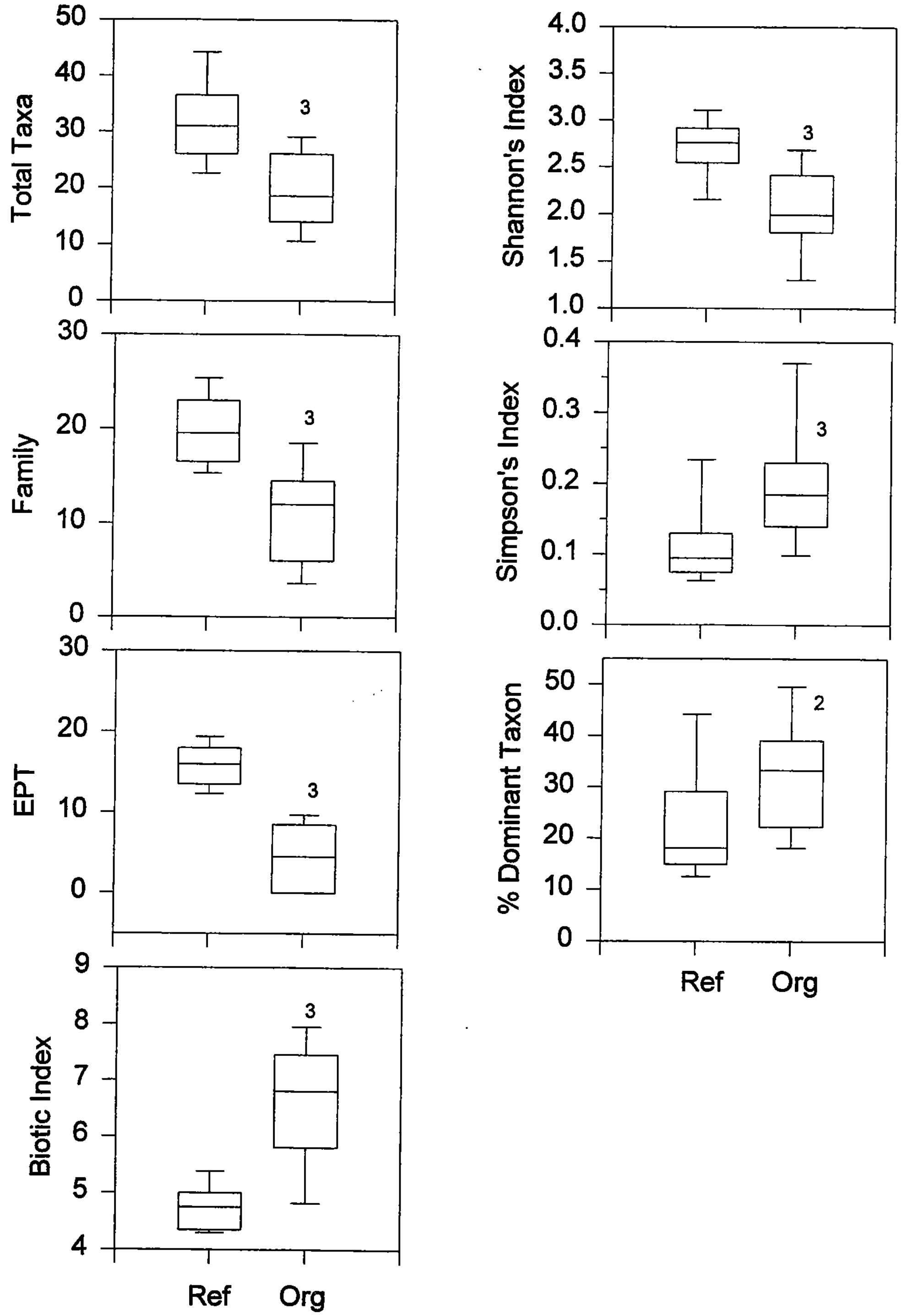


Fig. 20. Discriminatory power analysis for metrics examining reference vs. organically enriched sites, single habitat (cs flow 1995). Numbers indicate sensitivity, or ability to discriminate from reference condition, from 0 = no discrimination to 3 = greatest discrimination.

Part C Evaluation of Paired Metrics

Paired metrics are most often used to compare the invertebrate communities of two sites, one reference and one test. Less common is their use comparing the invertebrates of a test stream to an ideal reference condition. For all methods, the calculated similarity value is compared to an "impairment threshold" value to determine whether the test stream is considered impacted. There is a variety of ways to calculate how similar test sites are to references sites based upon taxa presence or absence, absolute numbers, or relative abundances (see Washington 1984 for a review) and we chose three of the most different approaches to evaluate: the Quantitative Similarity Index (QSI; identical to percentage similarity of Whittaker and Fairbanks 1958), the Coefficient of Community Loss (CCL; Courtemanch and Davies 1987) and percent model affinity (PMA; Novak and Bode 1992). Table 26 gives detailed descriptions and formulae. We calculated the paired metrics to compare reference to both habitat and organically degraded sites.

QSI - The impairment threshold for this metric was taken as the lower 10% of all values from a similarity matrix of all 1995 reference sites (Table 27). Very clear conclusions emerged from this analysis. First is that reference streams (REF) are not particularly similar. Mean similarity for all REF sites was 44%. The mean similarity for comparisons between REF and habitat degraded sites (HAB) was 45.3%. Thus habitat degraded sites were more similar to reference sites than were reference sites among themselves. No habitat degraded site had a value below the impairment threshold (Table 28). The mean similarity between REF and organically degraded sites (ORG) was 25.8 which is considerably less than the within reference value of 44.8. Five of the eight ORG sites would be below the threshold value using multihabitat data and six of eight sites using single habitat data (Table 28).

CCL - The impairment threshold for this metric was 0.80 which was recommended by Courtemanch and Davies (1987). The CCL metric would not classify any of the HAB sites as impaired but would classifying five of eight ORG with multihabitat data and six of eight ORG with single habitat data as impaired.

PMA - The QSI and CCL both compare a single reference to a single test site. A variation on this theme is the metric "percent model affinity," which compares a test stream to an ideal community, expressed as percent composition of seven major organism groups: Ephemeroptera, Trichoptera, Plecoptera, Diptera, Oligochaetes, Coleoptera, and other (Novak and Bode 1992). Our ideal reference condition was determined from our reference streams of 1995. We then compared the ideal stream community to the test streams using the QSI metric. Novak and Bode (1992), using data from an extensive (>300 sites) study in New York set 65% similarity as their threshold where values <65% were considered impaired. We used the value which was exceeded by 90% of the reference similarities which was 71% for multihabitat data and 72% for riffle (cs flow) habitats. Percent model affinity performed about equally to the other two paired metrics previously examined (Table 28). Using our threshold with multihabitat data, three of nine habitat degraded sites are classes as impaired, while six of eight organically degraded sites were considered impaired. Using data from only the riffles, three of nine HAB streams were below the threshold, while seven of the eight ORG would be considered impaired.

We conclude that the paired metrics performed about as well as many of the other metrics tested. Both metrics were good at detecting water-quality problem sites, but performed poorly at distinguishing habitat-degraded situations.

Table 26. Descriptions of the three paired metrics examined for this project.

Quantitative similarity Index (QSI)

QSI - Sum min(P*ia*, P*ib*)

where P*ia* and P*ib* are the relative abundance of species *I* at station A and B, respectively.
min(P*ia*, P*ib*) is the minimum possible value of species *I* at station A and B in terms of relative abundance.
QSI ranges from 0 (total different communities) to 100 (identical communities).

Coefficient of Community Loss (CCL)

CCL = *(a-c)/b*

where *a* is the numbers of taxa in the reference community, *b* is the numbers of taxa in the pollution affected community, and *c* is the numbers of taxa common to *a* and *b*.
CCL values exceeding 0.8 are indicative of excessively harmful change in those communities (Courtemanch and Davies 1987).
The RBP III (Plafkin et al. 1989) suggested the value 0.5 as the impairment threshold.

Percent Model Affinity (PMA)

PMA = Sum min(P_{ia}, P_{ib})

where P_{ia} is the relative abundance of one of seven faunal groups from the test site, P_{ib} is the relative abundance of the same faunal group in an ideal reference community. In this project the ideal community was determined from the 1995 reference sites and consisted of: Coleoptera 13%, Chironomidae 16.4%, Ephemeroptera 48.3%, Plecoptera 2%, Trichoptera 11.1%, Oligochaeta 2.6% and Other 6.7%.

Table 27. Similarities (QSI) for 1995 reference streams, multihabitat data.

	Starks	Deer	Woods Fk	E Fk Huzz	Crane Pond	Huzzah	Meramec	Maries	Swan	Big Sugar	Lindley	W Piney	Whet	Shawnee	N Jacks	Marble
Ltl Niag	0.596	0.585	0.508	0.552	0.544	0.356	0.504	0.481	0.377	0.339	0.395	0.548	0.522	0.484	0.522	0.593
Starks		0.601	0.583	0.460	0.509	0.314	0.552	0.537	0.433	0.321	0.448	0.592	0.534	0.531	0.576	0.551
Deer			0.506	0.386	0.411	0.381	0.522	0.425	0.429	0.399	0.368	0.480	0.481	0.393	0.460	0.468
Woods Fk				0.450	0.492	0.546	0.480	0.455	0.406	0.334	0.400	0.614	0.600	0.493	0.605	0.542
E Fk Huzz					0.549	0.390	0.363	0.390	0.427	0.304	0.308	0.550	0.513	0.548	0.496	0.553
Crane Pond						0.419	0.369	0.536	0.375	0.259	0.455	0.553	0.508	0.467	0.502	0.581
Huzzah							0.357	0.334	0.355	0.296	0.264	0.351	0.459	0.352	0.421	0.456
Meramec								0.538	0.435	0.241	0.297	0.448	0.440	0.590	0.511	0.518
Maries									0.296	0.231	0.317	0.413	0.367	0.489	0.572	0.557
Swan										0.315	0.258	0.416	0.404	0.343	0.383	0.356
Big Sugar											0.308	0.285	0.347	0.218	0.297	0.319
Lindley												0.446	0.428	0.302	0.326	0.331
W Piney													0.545	0.507	0.542	0.495
Whet														0.543	0.536	0.566
Shawnee															0.546	0.557
N Jacks																0.669

Table 28. Paired metrics, Ozark region, summer 1995. Asterisks indicate values showing impairment. Impairment thresholds are explained in text.

Stream		CS flow + nonflow			CS flow		
		QSI	CCL	PMA	QSI	CCL	PMA
REF	**HAB**						
Ltl. Niangua	Dry Aug.(C)	37	0.53	58*	41	0.46	65*
Starks	Greasy	44	0.46	12*	55	0.47	79
Deer	Cole Camp	37	0.25	63*	44	0.22	67*
Woods Fk.	Clark	53	0.31	72	49	0.33	74
E. Fk. Huzz.	Hutchins	48	0.39	84	39	0.44	81
Crand Pond	Crooked	33	0.51	86	37	0.29	74
Huzzah	Big Cr. (Iron)	49	0.37	80	51	0.44	76
Meramec	Indian	64	0.30	80	48	0.41	76
Maries	Ltl. Tavern	39	0.44	79	35	0.73	71*
REF	**ORG**						
Swan	Clear	10*	1.58*	26*	12*	2.42*	19*
Big Sugar	Turkey	19*	1.35*	36*	9*	2.70*	21*
Lindley	Piper	37	0.21	44*	19*	0.31	31*
W. Piney	Hominy	32	0.83*	51*	42	1.21*	70*
Whetstone	E. Fk. Whets.	8*	1.00*	26*	5*	1.43*	21*
Shawnee	Ltl. Lindley	25*	1.38*	36*	11*	1.45*	22*
N. Jacks	Dry Aug. (L)	39	0.56	72	15*	0.59	64*
Marble	Spring	28*	0.66	79	40	0.82*	79
Impairment threshold		30	0.80	71	30	0.80	72

Chapter Conclusion

Questions addressed in this chapter were as follows:

1. Which metrics were most sensitive for detecting habitat degradation?
The most sensitive metrics were the BI and Shannon's diversity index. EPT, Simpson's diversity index, and Family were intermediate, while Total taxa and % Dominant taxon were least sensitive.

2. Which metrics were most sensitive for detecting water quality problems?
The EPT, BI, and Shannon's diversity index were best, Family and Total taxa were intermediate, while % Dominant taxon and Simpson's diversity index were least likely to detect water quality impairment.

3. Which metrics were most sensitive for detecting impaired conditions?
Total taxa, Family, EPT, BI, and Shannon's diversity index were all excellent at detecting impairment. Simpson's diversity index and % Dominant taxon were somewhat less sensitive.

4. What was the difference in sensitivity between using single habitats versus using multihabitats?
Results were variable. In 1994, the multihabitat data performed somewhat better than single, while for 1995, the single habitat data was consistently, but not greatly, more sensitive. Overall multihabitat data showed some ability to discriminate 61% of the time, while single habitat data indicated sensitivity 67% of the time.

5. What was the difference in sensitivity between situations in the Ozark ecoregion versus the Prairie ecoregion?
Degradation was easier to detect in Ozark streams than in Prairie streams.

Chapter 9

INDEX DEVELOPMENT

INTRODUCTION

Biological criteria could be developed using one of the several metrics evaluated to this point. More common is the "multimetric approach" where metrics are aggregated into an index. Different metrics may relate different characteristics concerning stream integrity and, therefore, provide a more realistic picture of stream structure and function than a single metric. The procedure for developing an efficient index is to first select metrics with low variability, high sensitivity, and their ability to describe important but nonredundant characteristics of the invertebrate community. Variability and sensitivity of metrics have previously been examined. In this chapter we evaluate redundancy and choose appropriate metrics. We then develop the index, test its discriminatory power, and propose standards for impairment.

EVALUATION OF METRIC REDUNDANCY

The multimetric approach to biocriteria assumes each metric provides some unique information about the ecological situation being measured. Therefore, metrics selected to be part of an index should not measure identical characteristics of the benthic community. Metrics measuring the same feature of a community will be highly correlated. We evaluated the redundancy of the seven metrics using a combined dataset from spring and fall 1993, separated by region, and examined both single and multihabitat communities.

Multihabitat

Strong, significant correlations were found among the two diversity indices and the % Dominant taxon within each ecoregion as well as when data for the entire state was combined (Table 1). Additionally the metric Total taxa was significantly correlated with Family (r = 0.84), EPT (r = 0.77), and Shannon's diversity index (r = 0.73). However, within each region correlations between Total taxa and EPT to Shannon's diversity index decreased greatly (Table 1).

Single Habitat (cs flow)

Results for a single habitat were similar to the multihabitat analysis: strong associations among the diversity indices and % Dominant taxon, and between Total taxa and Family in every situation (Table 2). In contrast, the redundancy of Total taxa with EPT and Shannon's diversity index did not exist in all situations. Strong correlations existed between Total taxa and Family, and among the two diversity indices and % Dominant taxon.

METRICS CHOSEN FOR THE INDEX

Because Total taxa was more rigorous than Family, and Shannon's diversity index had always shown low variation and more sensitivity to impairment than did Simpson's diversity index and % Dominant taxon, we concluded that the metrics Family, Simpson's diversity index, and % Dominant taxon were redundant with

Table 1. Correlation coefficients (r) between metrics, multi-habitat, spring and fall, 1993.

	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson
A. Prairie and Ozark streams (N=61)						
Taxa						
Family	0.84					
EPT	0.77	0.71				
Biotic Index	-0.45	-0.39	-0.60			
Shannon	0.73	0.65	0.58	-0.46		
Simpson	-0.52	-0.46	-0.39	0.37	-0.93	
Dominant	-0.45	-0.38	-0.33	0.31	-0.88	0.97
B. Prairie streams (N=19)						
Taxa						
Family	0.67					
EPT	0.62	0.30				
Biotic Index	-0.18	0.15	-0.50			
Shannon	0.30	0.26	0.19	-0.39		
Simpson	-0.09	-0.08	-0.05	0.41	-0.95	
Dominant	0.00	-0.01	-0.04	0.40	-0.89	0.97
C. Ozark streams (N=42)						
Taxa						
Family	0.65					
EPT	0.47	0.46				
Biotic Index	0.00	0.04	-0.29			
Shannon	0.54	0.26	0.15	0.05		
Simpson	-0.33	-0.08	0.03	-0.11	-0.92	
Dominant	-0.25	-0.04	0.06	-0.11	-0.85	0.96

Table 2. Correlation coefficients (r) between metrics, cs flow habitat, spring and fall, 1993.

	Taxa	Family	EPT	Biotic Ind.	Shannon	Simpson
A. Prairie and Ozark streams (N=71)						
Taxa						
Family	0.87					
EPT	0.81	0.79				
Biotic Index	-0.18	-0.33	-0.39			
Shannon	0.83	0.76	0.69	-0.20		
Simpson	-0.70	-0.63	-0.55	0.12	-0.95	
Dominant	-0.67	-0.60	-0.53	0.09	-0.92	0.97
B. Prairie streams (N=19)						
Taxa						
Family	0.85					
EPT	0.45	0.51				
Biotic Index	0.37	0.18	-0.36			
Shannon	0.68	0.61	0.08	0.44		
Simpson	-0.50	-0.49	0.04	-0.41	-0.96	
Dominant	-0.48	-0.44	-0.01	-0.38	-0.92	0.97
C. Ozark streams (N=52)						
Taxa						
Family	0.81					
EPT	0.79	0.71				
Biotic Index	0.16	-0.01	0.00			
Shannon	0.79	0.66	0.71	0.07		
Simpson	-0.65	-0.52	-0.59	-0.14	-0.93	
Dominant	-0.61	-0.48	-0.51	-0.16	-0.89	0.95

other metrics and they were eliminated from further consideration as index metrics.

A successful index for a biocriteria program requires the integration of metrics that are of low variability in a natural situation, but highly sensitive to degradation. Each metric should provide unique information about the biota and the environment and be ecologically meaningful. Based on these criteria we selected four metrics—**Total taxa, EPT, Biotic Index, and Shannon's diversity index**—*to be included in the Stream* Condition Index (**SCI**). The SCI is a single value summary of the four metrics shown to be most appropriate for describing changes in the macroinvertebrate fauna (e.g., Barbour et al. 1996). The index should indicate values representing desired criteria, e.g., poor vs. good, or meeting vs. not meeting water quality standards.

NORMALIZATION OF METRICS INTO UNITLESS SCORES

To make the four metrics comparable and of equal importance in the SCI, all values were normalized to unitless values. We followed the suggestion of Barbour et al. (1992) and divided the range of each metric into one of three possible scores (Fig. 1). The lower quartile of the distribution of each metric from reference site data was used as the minimum value representative of reference conditions. For those metrics whose values decrease with impairment (Total taxa, EPT, Shannon's diversity index) any value above the lower quartile (25%) of the reference distribution received the highest score (5). For the BI whose values increase with impairment, any value below the upper quartile (75%) of the reference distribution received the highest score (5). Those sites in a lower condition have a score of 3, and a score of 1 represents the greatest deviation from the expected value.

Index scores were developed from summary statistics for different ecoregions, both single and multihabitat conditions, and for different seasons and years: 1) spring 1993—Prairie and Ozark ecoregions—multihabitat (Table 3); 2) spring 1993—Prairie and Ozark ecoregions, single habitat (cs flow; Table 4); 3) fall 1993—Prairie and Ozark ecoregions, multihabitat (Table 5); 4) fall 1993—Prairie and Ozark ecoregions, single habitat (cs flow; Table 6); 5) fall 1993—Prairie ecoregion, single habitat (nonflow; Table 7); 6) summer 1995—Ozark ecoregion, multihabitat (Table 8) and single habitat (cs flow; Table 9).

CALCULATION OF THE SCI

Using the metric scores from Tables 3-9, an SCI for each situation could be calculated by aggregating the scores of the metrics for each region. The minimum possible score for the SCI was 4 (equal to the number of metrics, while the maximum was 20 (4 metrics X the greatest possible score 5).

The discriminatory power of the SCI was then evaluated so as to determine the appropriate ranges for scores that are considered to be from impaired stream sites.

DISCRIMINATORY POWER OF THE SCI

Our three categories of streams: REF, HAB, and ORG from the Prairie and Ozark ecoregions for fall 1994 and Ozark ecoregion for summer 1995 were used to test the discriminatory power of the SCI. Comparisons were made using data from sites with identical habitat types.

First we compared REF and HAB sites from the fall dataset of 1994 in the Ozark region using scores from Table 3–multihabitat, which were developed from 1993 data. Results (Fig. 2) show no overlap of the interquartile ranges between REF

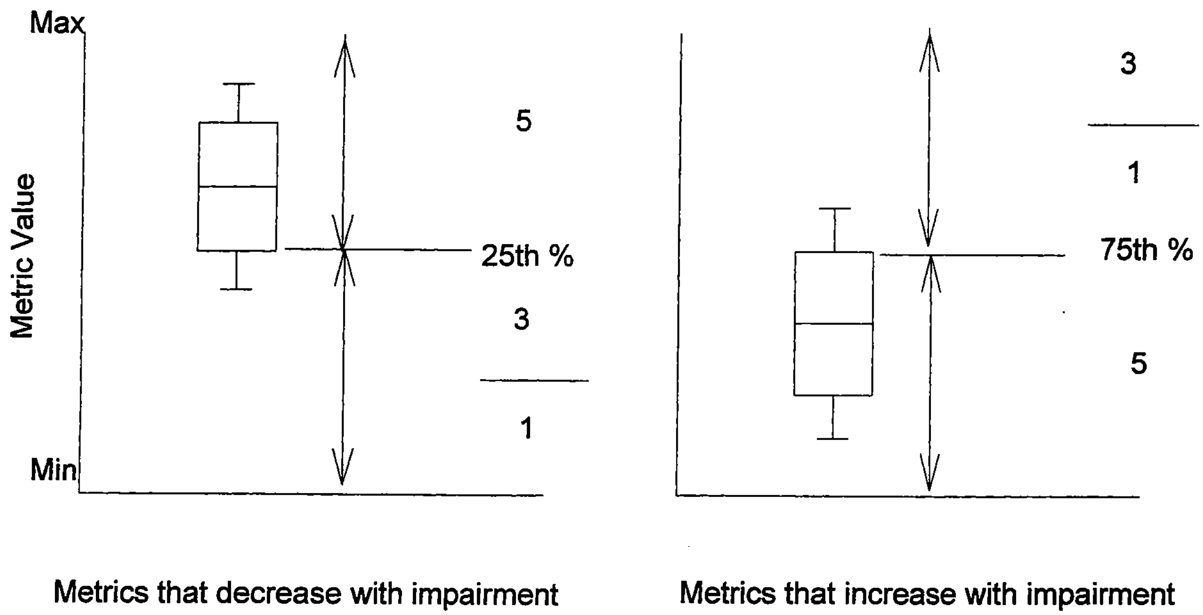


Fig. 1. An illustration of metric scoring procedure (after Barbour et al. 1992).

Table 3. Descriptive statistics and scores for the metrics for Spring Index Period, 1993. Based on multihabitat data (cs flow, nonflow and root mat).

Metric	Statistics					Scores		
	min.	25%	50%	75%	max.	5	3	1
Prairie (n=8)								
Taxa	22	32	36	42	47	>= 32	31-16	< 16
EPT	3	5	7	10	14	>= 5	4-3	< 3
Biotic Ind.	6.1	6.8	7.1	7.4	7.6	<= 7.4	7.5-8.7	> 8.7
Shannon	1.69	2.01	2.21	2.46	2.84	>=2.01	2.00-1.01	<1.01
Ozark (n=17)								
Taxa	38	47	53	57	63	>= 47	46-24	< 24
EPT	7	15	16	19	22	>= 15	14-8	< 8
Biotic Ind.	3.8	5.1	5.6	6.3	6.7	<= 6.3	6.4-8.1	> 8.1
Shannon	2.33	2.70	3.16	3.20	3.44	>=2.70	2.69-1.35	<1.35

Table 4. Descriptive statistics and scores for the metrics for Spring Index Period, 1993. Based on single habitat (cs flow) data.

Metric	Statistics					Scores		
	min.	25%	50%	75%	max.	5	3	1
Prairie (n=10)								
Taxa	10	15	18	20	29	>= 15	14-8	< 8
EPT	3	4	5	7	11	>= 4	3-2	< 2
Biotic Ind.	5.6	5.8	6.3	6.6	7.0	<= 6.6	6.7-8.3	> 8.3
Shannon	1.48	1.77	2.05	2.49	2.60	>=1.77	1.76-0.88	<0.88
Ozark (n=26)								
Taxa	15	22	27	28	36	>= 22	21-11	< 11
EPT	4	9	12	15	16	>= 9	8-5	< 5
Biotic Ind.	3.7	4.4	4.8	5.3	6.2	<= 5.3	5.4-7.77	> 7.7
Shannon	1.64	2.47	2.70	2.81	3.18	>=2.47	2.46-1.23	<1.23

Table 5. Descriptive statistics and scores for the metrics for Fall Index Period, 1993.
Based on multihabitat (cs flow, nonflow and root mat).

Metric	Statistics					Scores		
	min.	25%	50%	75%	max.	5	3	1
Prairie (n=11)								
Taxa	32	34	38	45	50	>= 34	33-17	< 17
EPT	7	9	10	12	16	>= 9	8-5	< 5
Biotic Ind.	5.5	5.9	6.2	6.5	6.8	<= 6.5	6.6-8.3	> 8.3
Shannon	1.85	2.40	2.61	2.69	2.86	>=2.40	2.39-1.20	<1.20
Ozark (n=25)								
Taxa	42	51	53	56	68	>= 51	50-26	< 26
EPT	11	14	15	18	22	>= 14	13-7	< 7
Biotic Ind.	4.3	4.6	5.5	5.9	6.7	<= 5.9	6.0-7.9	> 7.9
Shannon	2.34	2.93	3.13	3.31	3.45	>=2.93	2.92-1.46	<1.46

Table 6. Descriptive statistics and scores for the metrics for Fall Index Period, 1993.
Based on single habitat (cs flow) data.

Metric	Statistics					Scores		
	min.	25%	50%	75%	max.	5	3	1
Prairie (n=9)								
Taxa	10	12	15	19	28	>= 12	11-6	< 6
EPT	4	5	7	9	10	>= 5	4-3	< 3
Biotic Ind.	3.2	5.7	6.0	6.3	6.5	<= 6.3	6.4-8.1	> 8.1
Shannon	1.20	1.28	1.75	2.14	2.25	>=1.28	1.27-0.64	<0.64
Ozark (n=26)								
Taxa	16	21	26	29	35	>= 21	20-11	< 11
EPT	5	9	11	12	14	>= 9	8-5	< 5
Biotic Ind.	3.0	3.6	4.9	5.3	5.8	<= 5.3	5.4-7.7	> 7.7
Shannon	1.33	2.29	2.44	2.61	2.96	>=2.29	2.28-1.15	<1.15

Table 7. Descriptive statistics and scores for the metrics for Fall Index Period, Prairie, 1993. Based on single habitat (nonflow) data.

Metric	Statistics					Scores		
	min.	25%	50%	75%	max.	5	3	1
Taxa	15	20	22	25	32	>= 20	19-10	< 10
EPT	1	4	6	7	8	>= 4	3-2	< 2
Biotic Ind.	6.5	6.7	6.9	7.3	7.8	<= 7.3	7.4-8.7	> 8.7
Shannon	1.78	2.29	2.41	2.50	3.08	>=2.29	2.28-1.15	<1.15

Table 8. Descriptive statistics and scores for the metrics for Summer Index Period, Ozark, 1995. Based on multi-habitat (cs flow, nonflow, root mats) data.

Metric	Statistics					Scores		
	min.	25%	50%	75%	max.	5	3	1
Ozark (n=17)								
Taxa	45	58	63	67	71	>= 58	57-29	< 29
EPT	13	18	22	23	26	>= 18	17-9	< 9
Biotic Ind.	5.0	5.4	5.7	6.1	6.5	<= 6.1	6.1-8.0	> 8.0
Shannon	2.78	3.13	3.22	3.28	3.41	>=3.13	3.12-1.56	<1.56

Table 9. Descriptive statistics and scores for the metrics for Summer Index Period, Ozark, 1995. Based on cs flow habitat data.

Metric	Statistics					Scores		
	min.	25%	50%	75%	max.	5	3	1
Ozark (n=17)								
Taxa	22	30	33	35	46	>= 30	29-15	>= 15
EPT	11	13	17	18	20	>= 13	12-7	>= 7
Biotic Ind.	3.7	4.3	4.9	5.1	5.9	<= 5.1	5.2-7.6	<= 7.6
Shannon	1.99	2.57	2.87	2.94	3.18	>=2.57	2.56-1.29	>=1.29

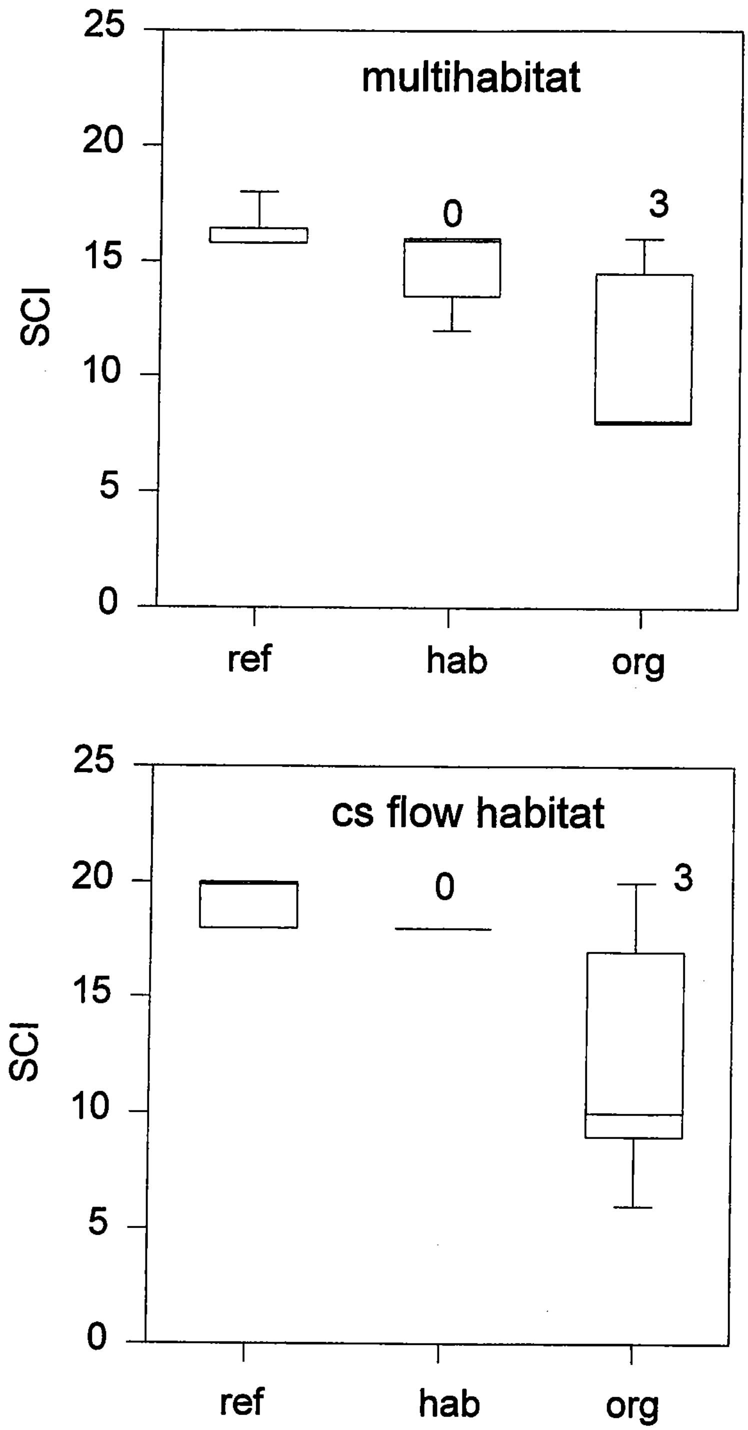


Fig. 2. Discriminatory power analysis of the Stream Condition Index (SCI) for the Ozark ecoregion; fall 1994 index period, using scores developed from fall 1993 data; numbers indicate ability to discriminate from Fig. 1.

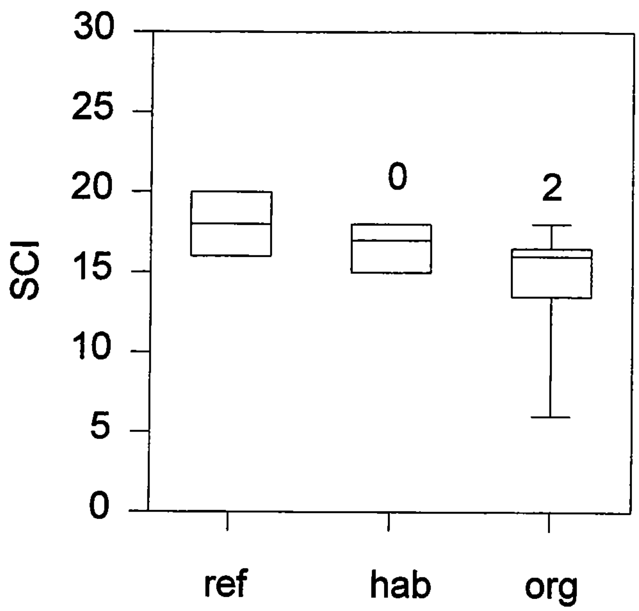


Fig. 3. Discriminatory power analysis of the Stream Condition Index (SCI) for the Prairie ecoregion; fall 1994 index period, nonflow habitat, using scores developed from fall 1993 nonflow habitat data set; numbers refer to ability to discriminate from Fig. 1.

and ORG sites which indicates an excellent ability to discriminate between these two groups. However, little discrimination was shown between REF and HAB sites. The median score of the HAB sites was equal to that of the REF indicating no impairment. Next we conducted a similar analysis except a single habitat (cs flow) based on scores from Table 4 was used. Results were the same as with multihabitat data: excellent separation of REF from ORG, little or no separation from HAB (Fig. 2).

Prairie sites were evaluated for the fall 1994 data, based upon scores from the fall 1993 nonflow data (Fig. 3). Nonflow habitat was chosen for the following reasons. In 1993, cs flow, nonflow, and rootmats were commonly selected, whereas in 1994 fs flow and nonflow were most common. Our analysis showed that nonflow was both the most representative habitat for the prairie region and possessed the widest number and variety of taxa.

Evaluation of fall 1994 data showed a fairly good distinction between REF and ORG (a value of 2). No discrimination could be shown between REF and HAB.

When comparing the REF to a group of four "impaired" sites (see details of previous results, Ozark fall 1994 for definition of impaired) there was total separation between types for both multihabitat (Fig. 4) and cs flow (Fig. 4) indicating excellent discriminatory ability of the SCI.

We further tested the discriminatory power of the SCI using the summer 1995 single habitat (cs flow; Fig. 5). The descriptive statistics and scores were from the same 1995 REF streams (Table 8). There was good ability to discriminate HAB streams (score = 2), and excellent ability to discriminate ORG streams (score = 3). Apparently having the ability to set REF conditions from the same year and season as the test conditions further increases the ability to reduce natural variation and, therefore, be able to detect impairment.

A further analysis combined REF and degraded sites data from fall 1994 and summer 1995 in order to increase sample size (Fig. 6). Metric scores based on fall 1993 without transition sites (Tables 5 and 6) were used. No overlap of any interquartile ranges were found for the REF–ORG comparisons in either the multihabitat or single habitat plots. The REF-HAB comparisons were less clear cut: the medians were the same for multihabitat comparison, but there was better separation for the single habitat.

We conclude from these several analyses that the SCI had excellent ability to discriminate REF sites from both ORG degraded sites and IMP sites but not a good ability to detect habitat problems. This is not that surprising, because the metrics used in the SCI, when used individually, also had difficulty detecting just habitat degradation.

Given that the SCI is able to detect impairment in many situations in both the Ozark and Prairie ecoregions it is now appropriate to classify the degree of impairment. This may be done in a number of ways and we will suggest only one.

Ordinal Rating Scale

We suggest a three level classification of *no impairment, impaired,* and *highly impaired* based on the following criteria. Reference sites SCIs for all seasons and years typically had their lower 25th percentile above a score of 16 (Figs. 2-6), and scores of 16-20 were selected as no impairment. Sites known to be impaired had a median at about a score of 10, and the range of 10-14 was selected as impaired. Scores of 4-8 were considered highly impaired (Table 10).

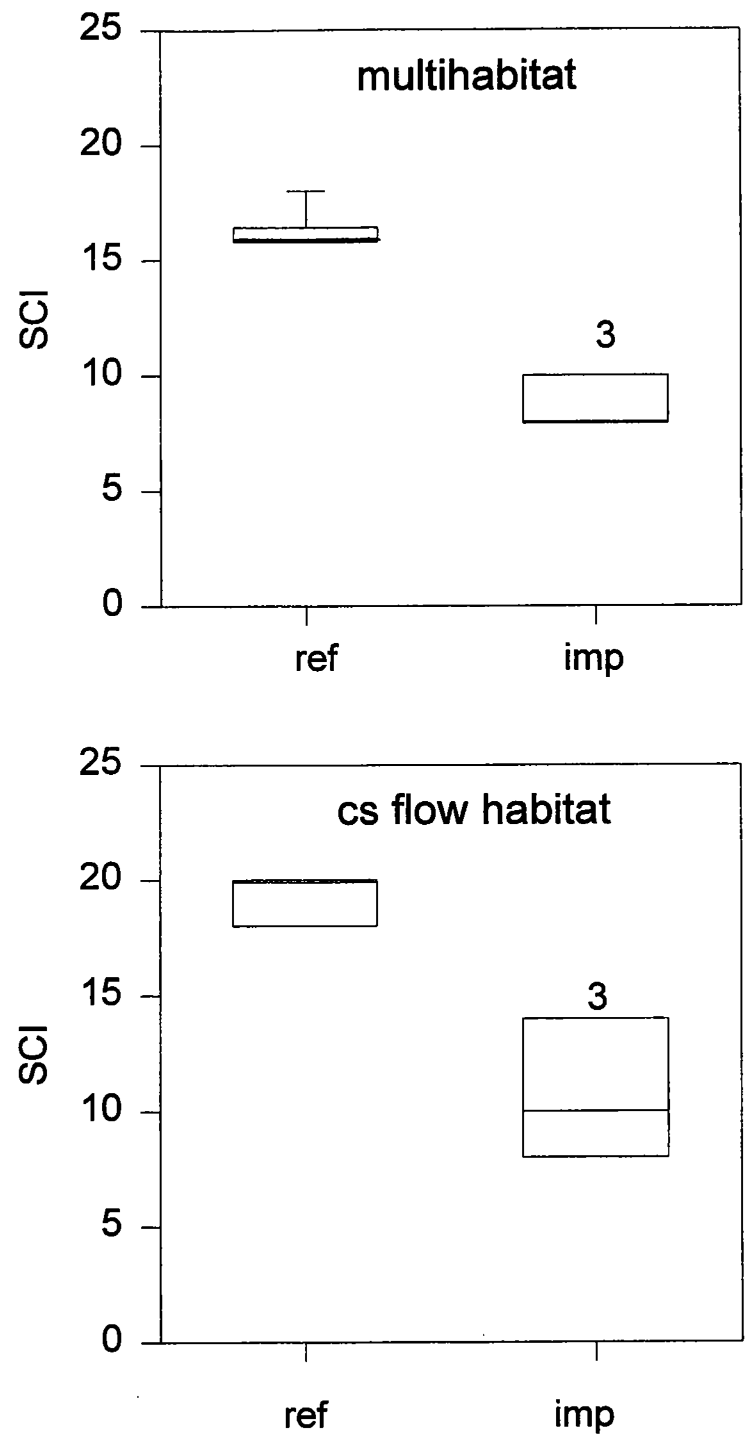


Fig. 4. Discriminatory power analysis of the Stream Condition Index (SCI) for a set of impaired sites from the Ozark ecoregion; fall 1994 index period, using scores developed from fall 1993 data; numbers refer to ability to discriminate from Fig. 1.

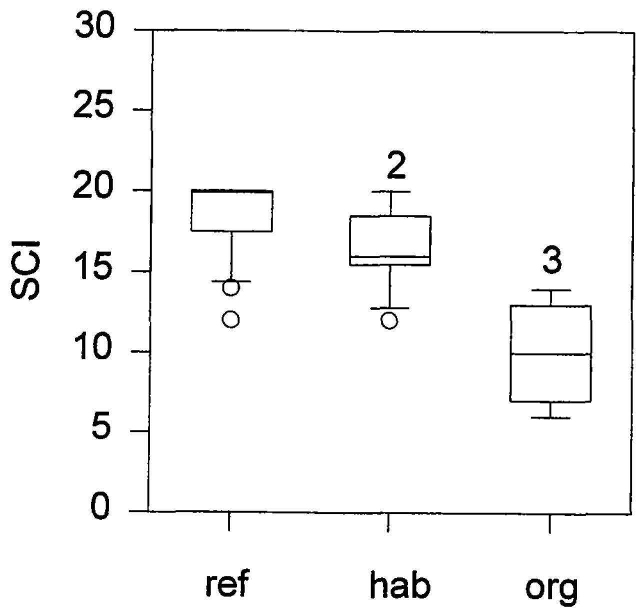


Fig. 5. Discriminatory power analysis of the Stream Condition Index (SCI) for the Ozark ecoregion; summer 1995 index period, cs flow habitat, using scores developed from summer 1995 data; numbers refer to ability to discriminate (see Fig. 1).

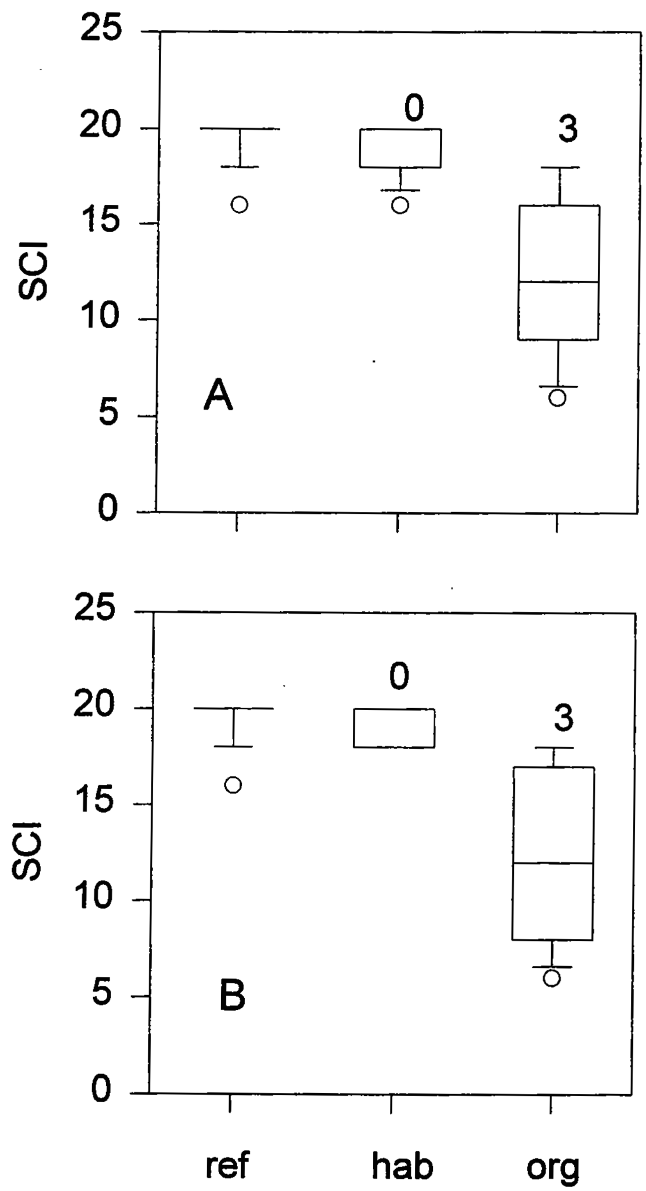


Fig. 6. Discriminatory power analysis of the Stream Condition Index (SCI) for the Ozark ecoregion; combined fall 1994 and summer 1995 index period, using scores developed from fall 1993 data; numbers refer to ability to discriminate (see Fig. 1); A = multihabitat, B = single habitat.

Table 10. Suggested rating scale for a Missouri Stream Condition Index.

Rating	SCI-Score
No impairment	16-20
Impaired	10-14
Highly impaired	4- 8

Chapter 10

THE UTILITY OF HABITAT-SPECIFIC SAMPLING

INTRODUCTION

A successful bioassessment program is one which effectively reduces the natural variation of the biological system so as to be able to detect impairment. Variation is present in both spatial and temporal dimensions. We know that benthic communities differ due to geographical location (Corkum 1989). Within a watershed different sized streams support different communities (Vannote et al. 1980). At any one location community structure differs according to microhabitat (Rabeni and Minshall 1977) and changes over time due to unique life cycles of each taxon (Hynes 1961). We followed the lead of the EPA by dividing the state into ecoregions (Omernik 1995) to control large-scale geographic variation; watershed level variation was controlled by our selection of streams of a particular and comparable size, and local variation was addressed by sampling over a short time period and at well-defined habitats within a stream site.

Two philosophies regarding sampling a site for bioassessment purposes are prevalent. The EPA recommends single habitat sampling to limit the effect of interhabitat variation on assessment (Plafkin et al. 1989), while Lenat (1988) and others recommend collecting from all major habitats and then compositing the sample. The multihabitat approach is sometimes favored because it is believed that communities from different habitat types may be differentially affected by impairment, and a single habitat analysis may miss these effects, while the single habitat school regards multiple sampling as redundant and a waste of resources (Parsons and Norris 1996). The approach used in this study differed from most others in that while we collected from many different habitats, we did not composite the individual samples into a single site sample. We analyzed each habitat separately, which allowed us to develop indices based upon single habitats or any combination of habitats. Even when we used several habitats, our approach was different than most others because each of the habitats is considered to be equally represented—and each is given equal "weight" in the analysis. We feel this approach is more standardized and more appropriate than the often used "representative sample from all habitats" or the "sample in proportion to the availability of habitats," which are often used in multihabitat sampling.

If the multihabitat approach of using invertebrates from a variety of habitat types at a site in a biocriteria program is being considered, it is necessary to evaluate the community of each habitat in terms of its similarity of structure and its usefulness to each metric. This chapter centers on comparing invertebrate communities from different habitats within a region and comparing communities from a single habitat between regions. Data analyzed here were from spring and fall 1993 surveys of all reference streams.

A visualization of the similarities among community associated with various habitat types within a region was afforded by ordination of reference streams sampled in 1993. In the Ozark region during spring (Fig. 1) some habitats had quite distinct communities. For example, rootmat communities were grouped away from all other communities with very little overlap.

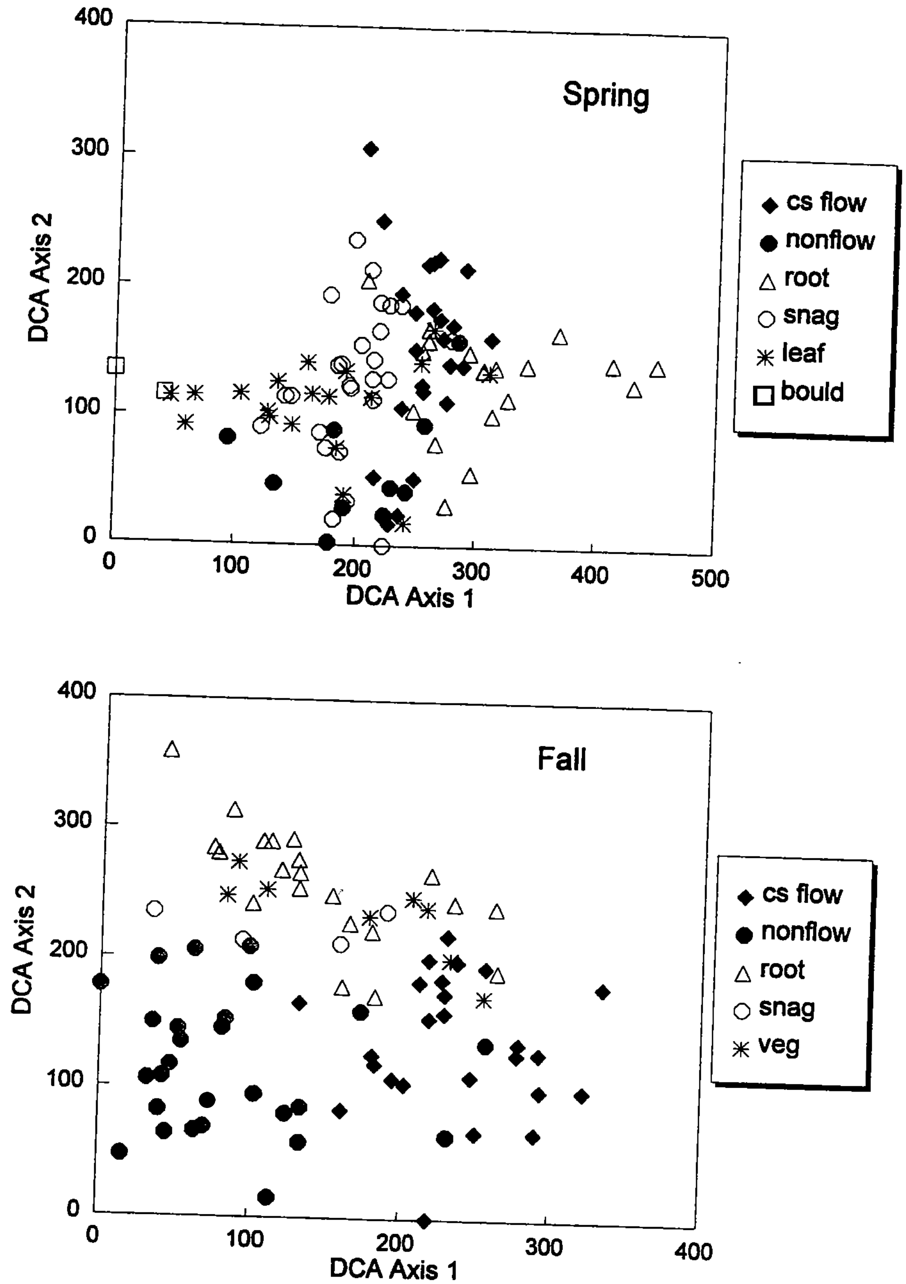


Fig. 1. Ordination of benthic invertebrate communities collected from different habitat types in reference streams, Ozark ecoregion, spring and fall 1993.

Other communities overlapped only slightly—cs flow vs. leaf packs or snags vs. nonflow. In the Ozark region in the fall there was some overlap among communities, but each habitat type tended to remain separate (Fig. 1). Nonflow had very little overlap with any other community. Vegetation and rootmat communities had the most similar structure.

In the prairie region streams during spring individual habitats were less separated than were the Ozark communities, although nonflow communities were distinct from snags and rootmat communities were distinct from fs flow (Fig. 2). In the prairie region during fall each habitat tended to group in its own cluster, but there was considerably more interspersion of habitat types than in the Ozarks (Fig. 2). Leafpacks, rootmats, and snags were highly interspersed, while fs flow and nonflow appeared to separate themselves from other habitat types.

Overall, Ozark stream sites had more distinct habitat-specific communities than those from the prairie, while habitats involving organic matter, rootmats, vegetation, and snags tended to be similar. This analysis suggests that, within the same ecoregion, communities collected from the same habitat at different sites are usually more similar than those collected from different habitats at an individual site. Similar conclusions have been made by Brown and Brussock (1991).

ANALYSIS OF METRIC VALUES FOR COMMUNITIES FROM INDIVIDUAL HABITATS, BY REGION

Considerable variance was shown among metrics evaluated for each habitat within a region. In the prairie region, the means for all metrics were significantly different among habitats except for EPT and Total taxa (ANOVA, $P < 0.05$) both in the spring and in the fall (Figs. 3 and 4). In Ozark streams all metrics showed significant differences ($P < 0.001$) in the spring (Fig. 5) but only the EPT and BI were significantly different among habitats in the fall (Fig. 6). When comparing the three major habitats of cf flow, nonflow, and rootmat which are typically present in Missouri streams: Total taxa, EPT, and BI were significantly different among habitats ($P = 0.05$). Boulder habitat was only sampled at a few sites, but nevertheless was so unusual in community structure that it probably should be omitted from further consideration.

Evaluation of Benthic Invertebrate Communities of Individual Habitats Between Regions

This section examines how communities from the same habitat type differ between regions (Figs. 7-9). We first analyzed the four most common habitat types using the spring 1993 reference stream data (Fig. 7).

CS Flow

The sites were entirely separated by region except for a single site (Fig. 7) which was a transition site (Site 16, see Fig. 1, Chapter 3). Prairie sites were much more similar to each other than were the Ozark sites.

Nonflow

Communities from nonflow habitats were not well separated by region (Fig. 7). Prairie sites were very similar to each other, much more so than the Ozark communities. Rootmats and snag communities also were generally separated by region, but with some interspersion of sites.

Three of the most common habitat types were used to compare between regions (Fig. 8) using the fall 1993

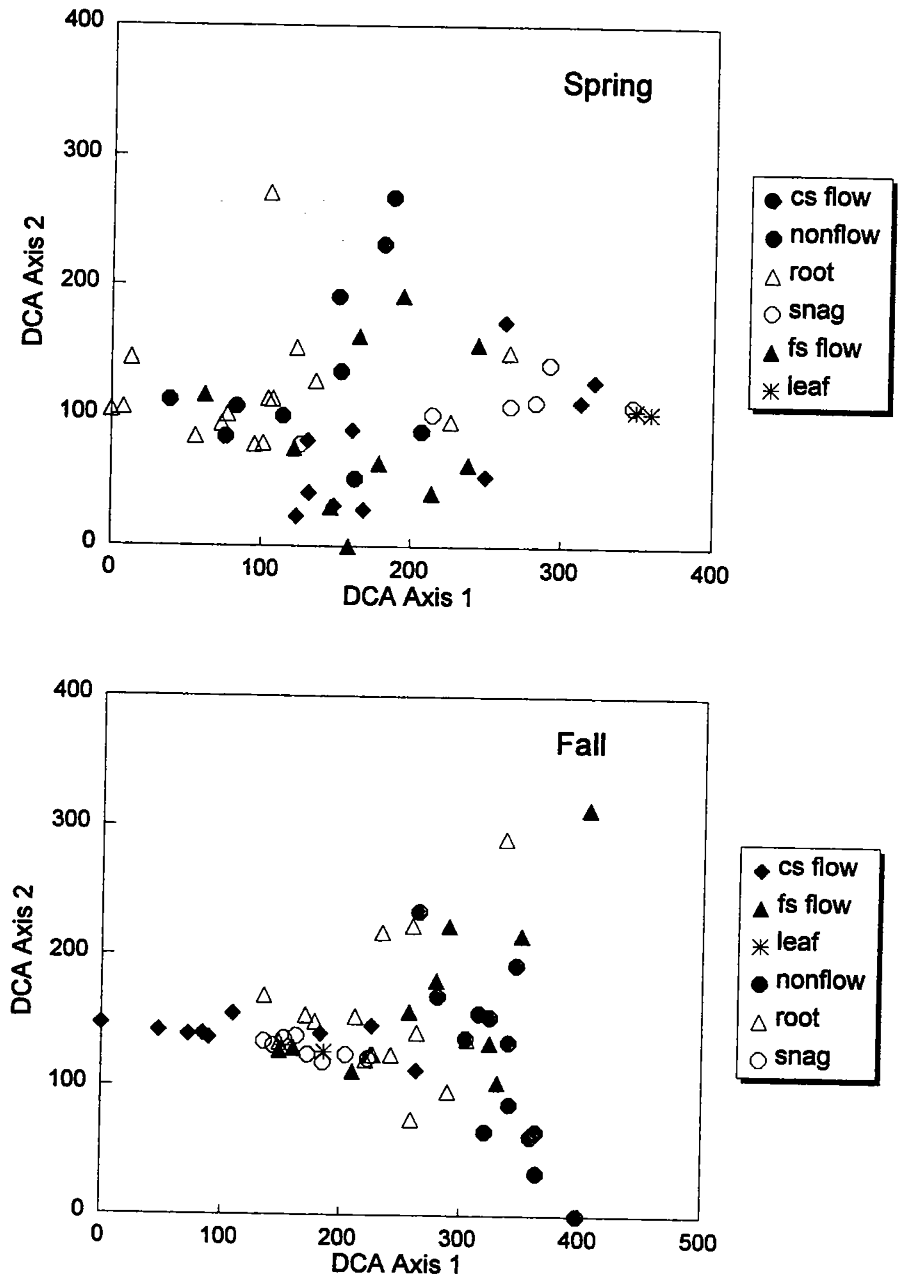


Fig. 2. Ordination of benthic invertebrate communities collected from different habitat types in reference streams, prairie ecoregion, spring and fall 1993.

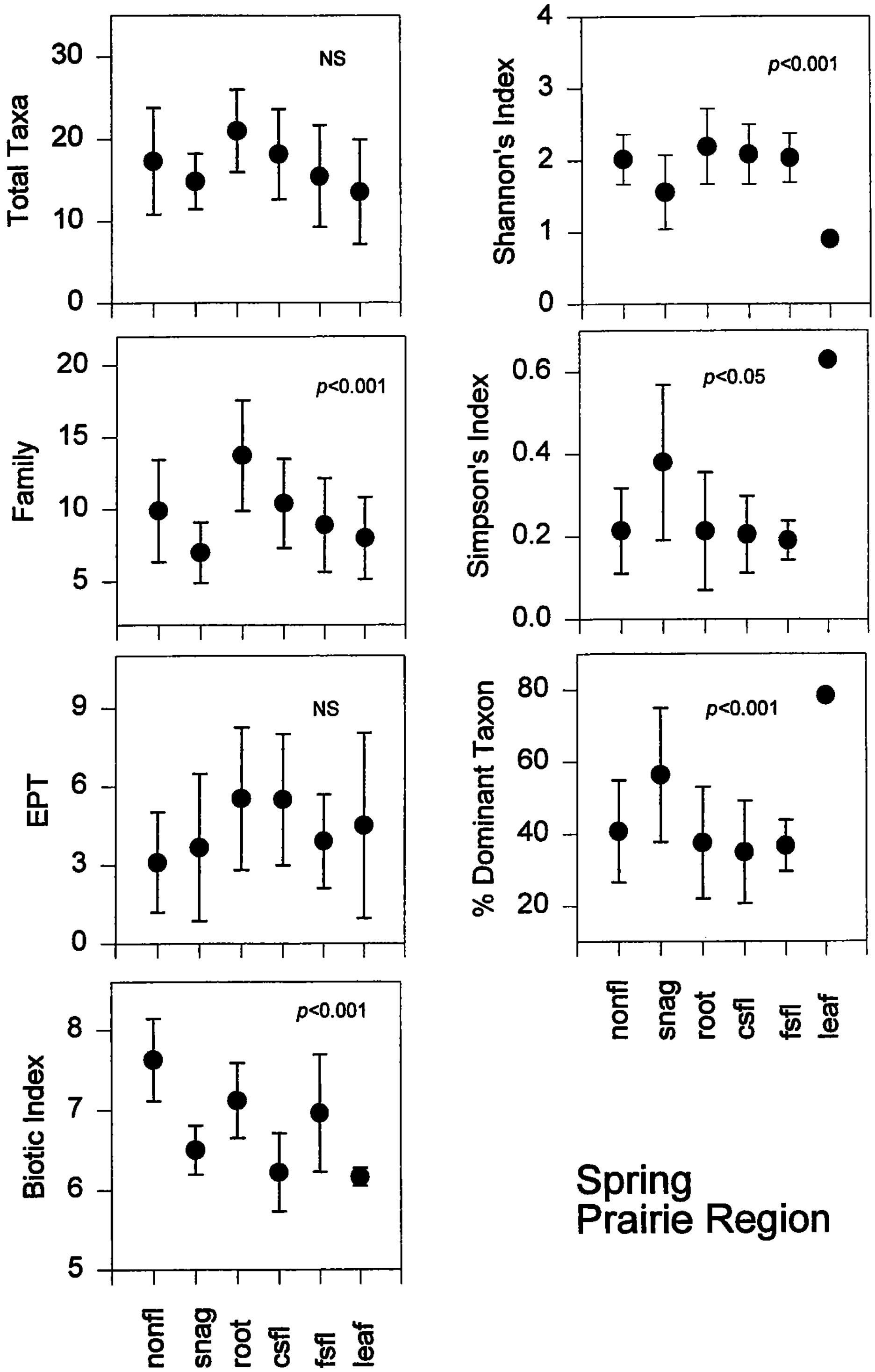


Fig. 3. Mean metric values (±sd) for benthic invertebrate communities collected from different habitat types in prairie reference streams, spring 1993.

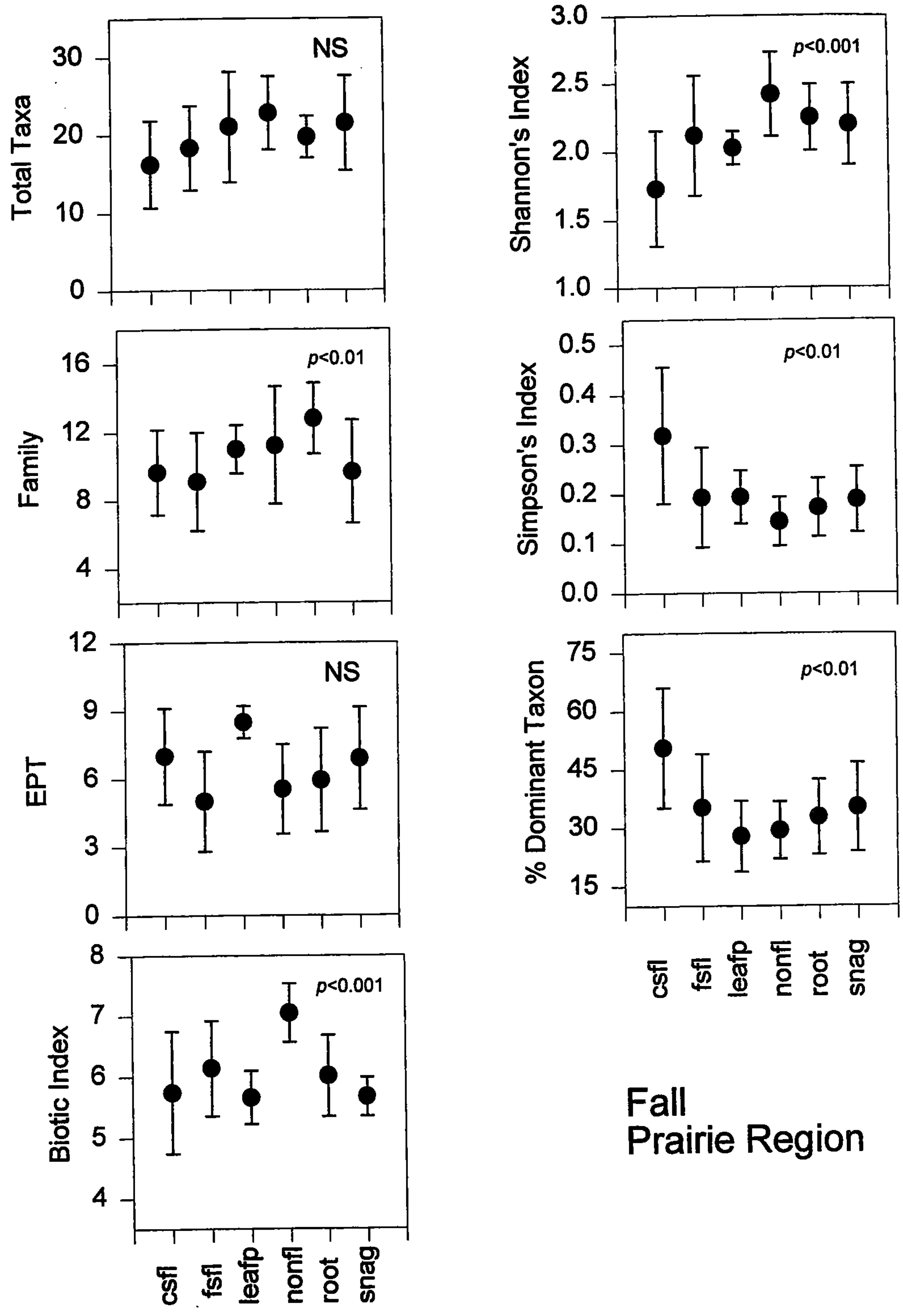


Fig. 4. Mean metric values (±sd) for benthic invertebrate communities collected from different habitat types in prairie reference streams, fall 1993.

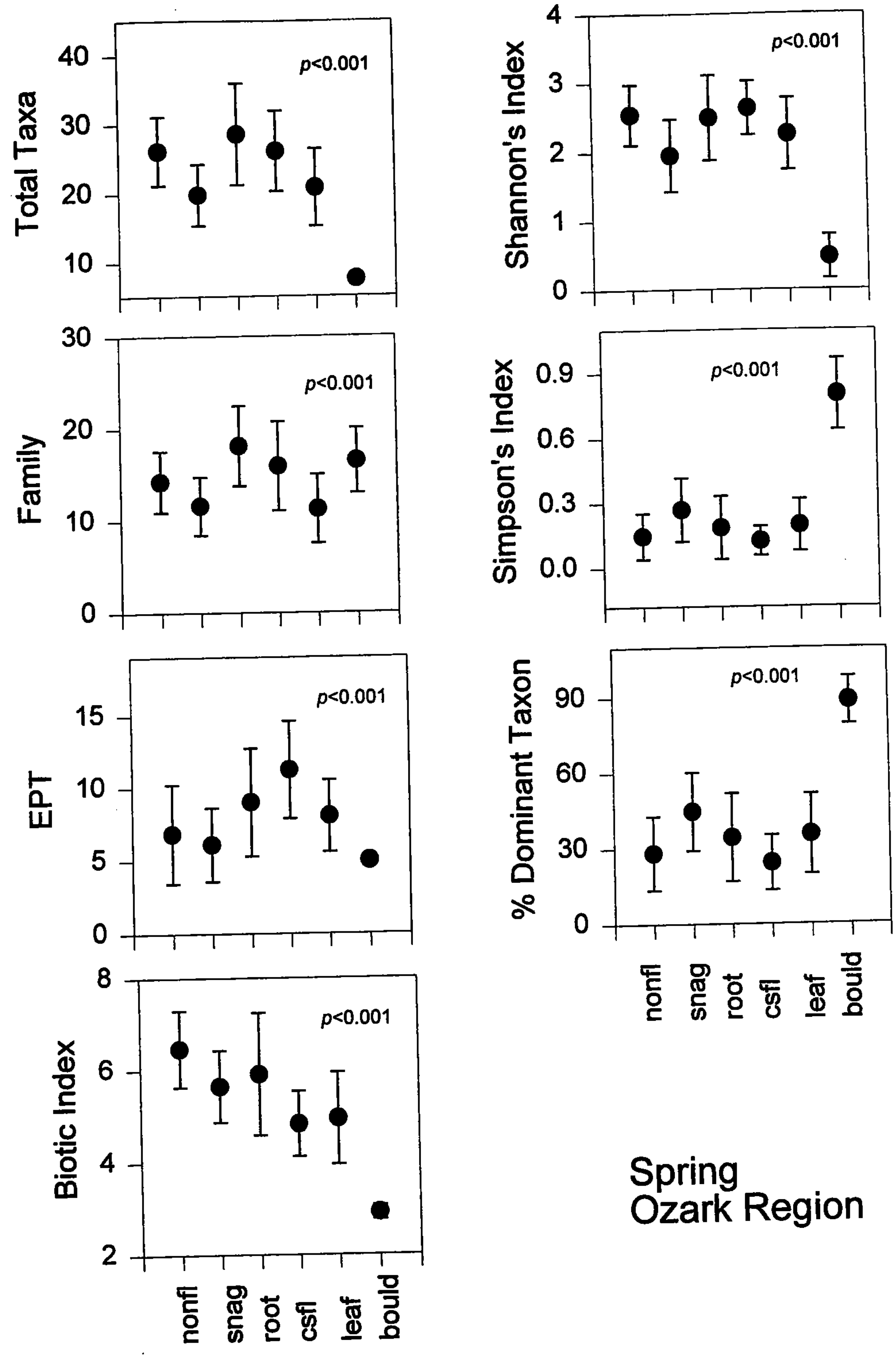


Fig. 5. Mean metric values (±sd) for benthic invertebrate communities collected from different habitat types in Ozark reference streams, spring 1993.

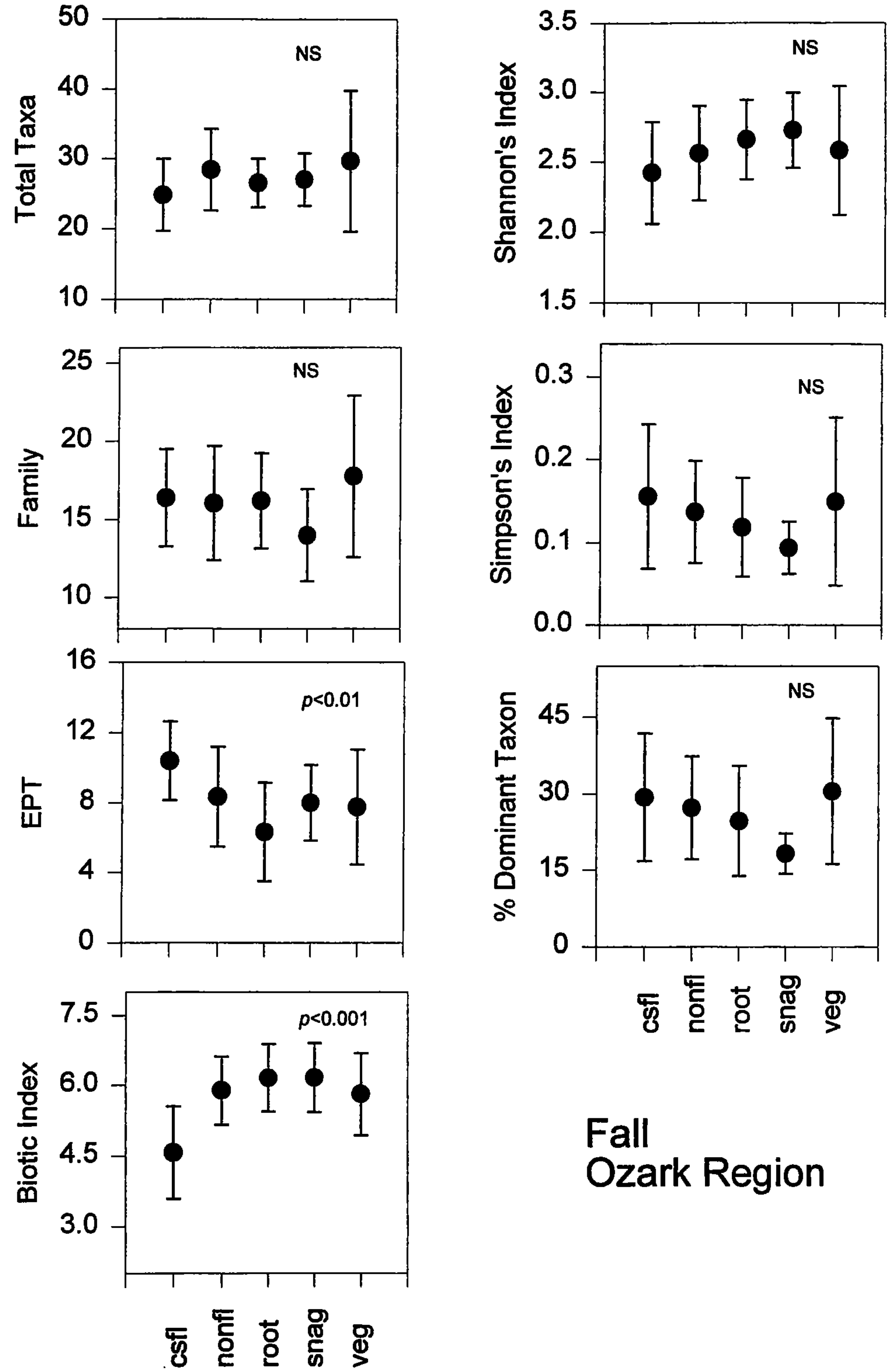


Fig. 6. Mean metric values (±sd) for benthic invertebrate communities collected from different habitat types in Ozark reference streams, fall 1993.

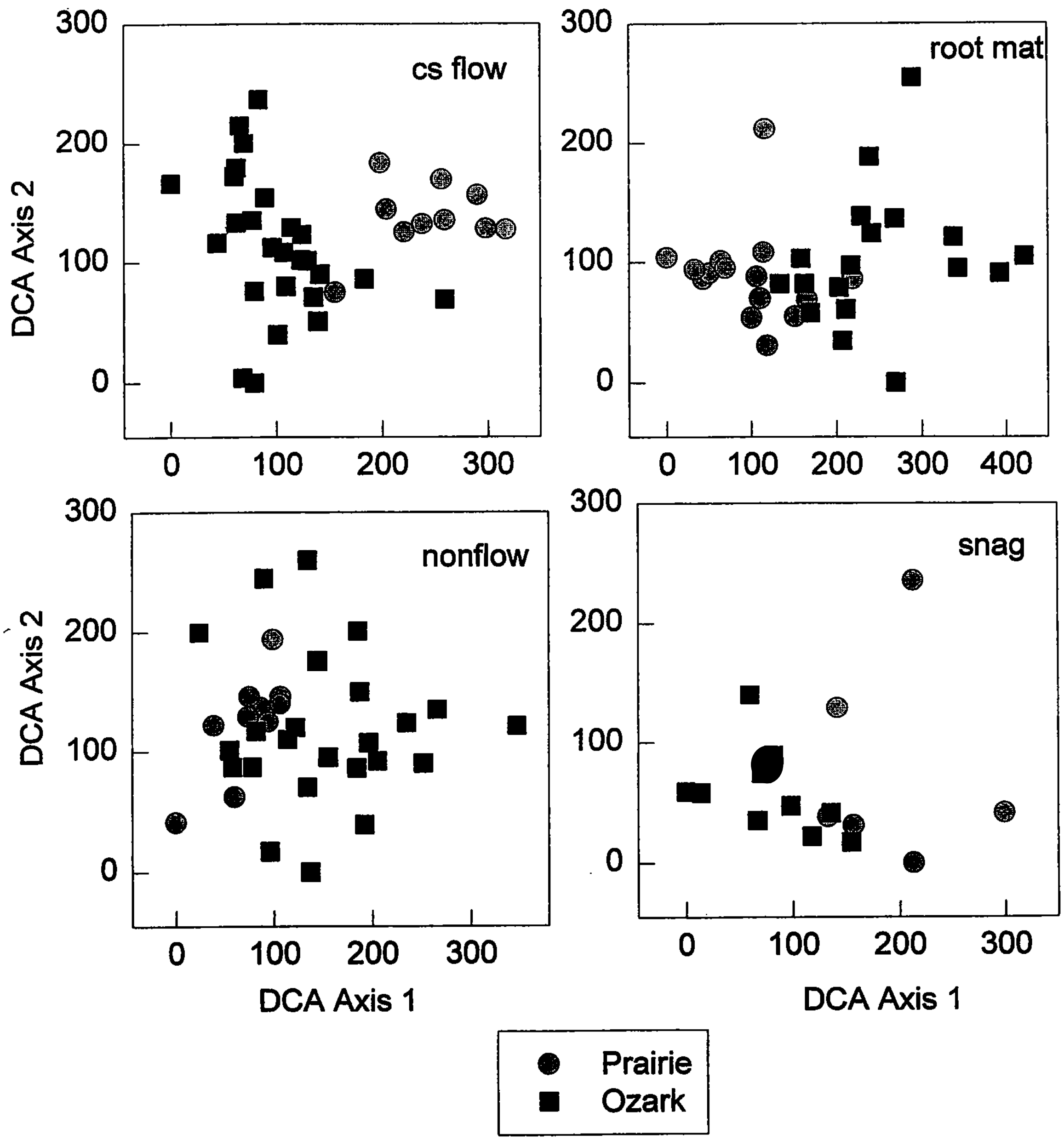


Fig. 7. Ordination of statewide benthic invertebrate communities from four habitat types, spring 1993.

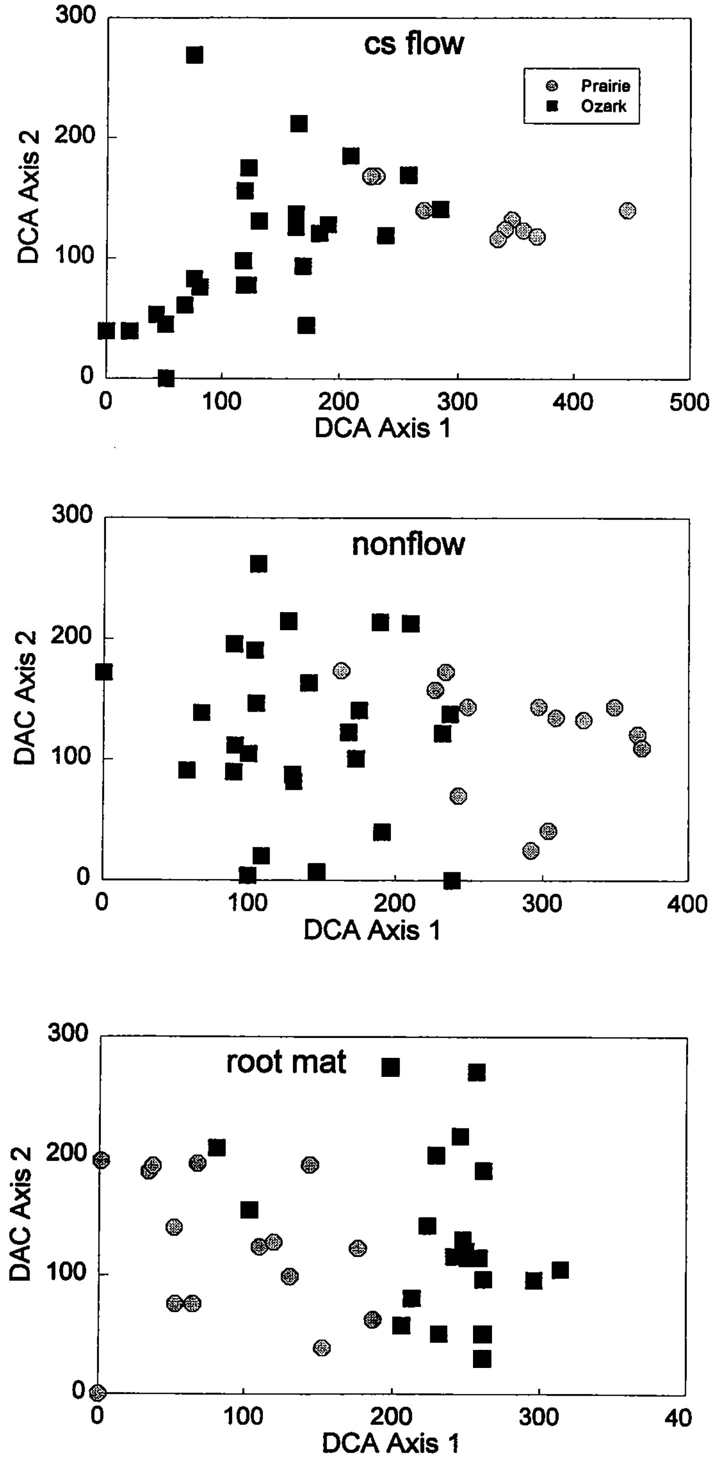


Fig. 8. Ordination of statewide benthic invertebrate communities from three habitat types, fall 1993.

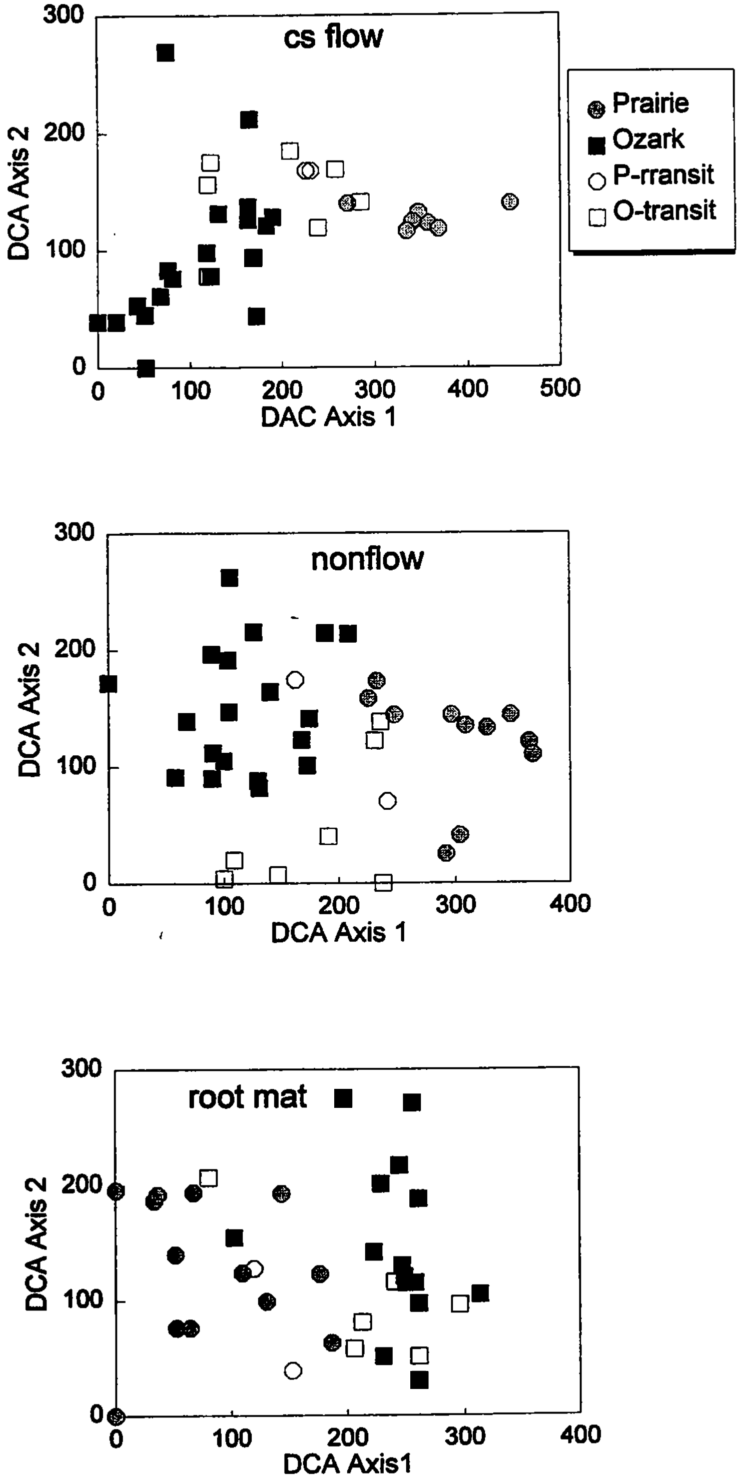


Fig. 9. Ordination of statewide benthic invertebrate communities from three habitat types, fall 1993. Figure differs from Fig. 8 by indicating the geographic transitional streams.

reference stream dataset. The same ordinations but with the geographical transition streams indicated are presented in Fig. 9.

CS Flow

This community was very distinctive in the prairie region (Fig. 8), especially after considering the transition streams (Fig. 9).

Nonflow

This community was not so distinctive (clumped) in either region, although the separation between regions was quite good (Fig. 8). Again the distinction becomes even greater when transitional streams are indicated (Fig. 9).

Rootmat

Communities from this habitat were regionally distinct, although there was a large variation within each region (Fig. 8).

Overall, region was an important factor in structuring communities from each habitat. That is, factors associated with the region are more important than any particular habitat type in structuring taxa composition. Prairie communities were generally much more similar to each other than were Ozark sites for any particular habitat type. The communities were most different by region in the cs flow habitat. This probably reflects the influence of the differing geology and soils between the two regions that result in a different physical habitat that we classified as cs flow.

Analysis of Metrics Between Regions, by Habitat Type

Spring

An evaluation of metrics developed for the cs flow habitats showed significant differences for all six metrics tested (Table 1). Nonflow habitats showed five of six metrics significantly different between regions, rootmats three significant tests, and snags two significant results.

Fall

Seven metrics were evaluated. For cs flow every metric was significantly different between regions (Table 2). For the nonflow habitat Total taxa, Family, EPT, and the BI were all significantly different between regions. For rootmats, all metrics were significantly different between regions except EPT and BI.

CONCLUSIONS

These results indicate a hierarchical influence of invertebrate distribution. At the largest scale, regions were more influential than habitats, because invertebrates collected from the same habitats grouped into distinct regional assemblages. Within a particular region habitats were more important than were sites because communities collected from the same habitat at different sites were more similar than those collected from different habitats at a particular site.

The results have practical significance as they lend credence to the ecoregion approach and our ecoregion delineations, as well as suggesting caution to make sure variance due to habitat differences does not increase the difficulty of detecting perturbations.

Because each habitat tended to possess a unique fauna, a multihabitat approach would give a more comprehensive view of the entire community at any particular site. This would be important if communities of different habitats were differentially affected by perturbation. A single habitat approach would certainly reduce sample variation. The multihabitat approach is only appropriate if comparisons are made using habitats in common from all sites.

Table 1. Mean metric values of the reference sites within a ecoregion, spring 1993. Differences in means between ecoregions tested by t-test: NS, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001.

Region	N	Taxa	Family	EPT	Biotic Ind	Shannon	Simpson	Dominant
				CS Flow				
Prairie	10	18.1	10.4	5.5	6.2	2.08	0.20	35
Ozark	26	26.1	15.9	11.2	4.8	2.62	0.13	24
		***	**	***	***	***	*	*
				Nonflow				
Prairie	10	17.3	9.9	3.1	7.6	2.02	0.21	41
Ozark	24	26.2	14.3	6.8	6.5	2.54	0.15	28
		**	**	***	***	***	NS	*
				Root mats				
Prairie	15	20.9	13.7	5.5	7.1	2.20	0.21	38
Ozark	18	28.6	18.1	9.0	5.9	2.49	0.18	34
		**	*	**	***	NS	NS	NS
				Snag				
Prairie	6	14.8	7.0	3.7	6.5	1.56	0.38	56
Ozark	10	19.8	11.6	6.1	5.7	1.91	0.27	45
		*	**	NS	**	NS	NS	

Table 2. Mean metric values of the reference sites within a ecoregion, fall 1993. Differences in means between ecoregions tested by t-test: NS, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001.

Region	N	Taxa	Family	EPT	Biotic Ind	Shannon	Simpson	Dominant
				CS Flow				
Prairie	9	16.2	9.3	7.0	5.7	1.73	0.32	50.6
Ozark	26	24.8	16.3	10.4	4.6	2.42	0.16	29.3
		***	***	**	**	**	**	**
				Nonflow				
Prairie	13	22.8	11.2	5.5	7.1	2.42	0.14	29.3
Ozark	26	28.4	16.0	8.3	5.9	2.56	0.14	27.3
		**	***	**	***	NS	NS	NS
				Root mats				
Prairie	15	19.7	12.8	5.9	6.0	2.25	0.17	32.7
Ozark	21	26.5	16.2	6.3	6.2	2.66	0.01	24.7
		***	**	NS	NS	***	**	**

Chapter 11

EVALUATING THE ADEQUACY OF FIELD SAMPLING

INTRODUCTION

The Rapid Bioassessment Protocols attempt to use cost-saving techniques so that a large amount of data can be accumulated in a short period of time (Plafkin et al. 1989). Yet cutting corners during field sampling could undermine the accuracy of all subsequent data analyses and conclusions. Most often, a single sample from a single location is taken. It is assumed that a single sample is sufficient because sampling error is reduced by taking samples from several habitats or many subsamples from several habitats. It is assumed a single location is sufficient because the random choice of a location is considered representative of much of the stream. We tested the assumption of the adequacy of our sampling within a single site by sampling twice at the same site at several streams in spring and fall 1993. We then tested the assumption of sampling a single location by sampling several contiguous sites on each of several streams and comparing the results in 1994.

EFFECT OF DOUBLING THE SAMPLING EFFORT

We evaluated the reproducibility of results from a particular stream by comparing metrics derived from two sets of collections from the same site taken the same day in nine reference streams in spring 1993 (Table 1) and eight streams in fall 1993 (Table 2). To examine how similar duplicate collections were for any particular metric, we simply divided the smaller of the two values by the larger, and termed this % Reproducibility (R%).

$$R\% = 100 \text{ Min } (M_1, M_2)/\text{Max } (M_1, M_2)$$

where M_1 and M_2 are the values for a metric from the first and second sample.

Spring

Reproducibility was high and consistent for all metrics except those composed of ratios (Table 1). If a somewhat arbitrary acceptable level of reproducibility is set at 75%, the seven other metrics appear highly reproducible. These seven metrics are the same ones previously selected because of their ability to discriminate between regions and for their low variation.

Fall

For the fall data only the seven best metrics were examined. By omitting the ratio metrics, and using in the analysis only those habitats in common—in this case cs flow, nonflow, and rootmat—all metrics from every stream except Simpson's diversity index (2 streams) and % Dominant taxon (four streams), were above our 75% cutoff considered to be very reproducible (Table 2).

EFFECT OF SAMPLING AT MULTIPLE SITES

A further evaluation of the number of replicate samples needed at a site was carried out during spring of 1994. Eight streams were selected: two from the prairie ecoregion, five from the Ozark ecoregion, and one Lowland site (Fig. 1, Table 3). Three or four sites along a 2-km stretch of

Table 1. Reproducibility (%) (see text for formula) of metrics for duplicate sites from nine reference streams in the three ecoregions for spring 1993. All available habitats were included in the analysis. Reproducibilities < 75% are in bold.

	Prairie				Ozark				Lowland
Metrics	Long B. Pl.	E.F.Crook.	Dry Wood	M.Fabius	Whitewater	Deer Ck	Ltl.Maries	Ltl. Black	Huffstetter
Taxa	96.9	100.0	81.8	**68.6**	94.0	93.7	82.1	95.3	84.6
Family	88.9	90.9	90.5	**69.7**	100.0	94.4	77.3	91.4	**71.4**
EPT	**66.7**	80.0	83.3	**58.8**	82.4	95.0	83.3	90.0	100.0
Biotic Index	99.6	95.4	95.9	78.6	87.9	94.6	90.8	95.7	97.4
Shannon Index	78.8	97.2	88.0	98.8	87.0	95.9	89.0	96.0	85.9
Simpson Index	81.9	82.7	**74.0**	91.7	**55.9**	78.2	**60.3**	85.7	81.1
Dominant	89.7	83.5	83.9	93.0	**61.3**	**70.5**	**52.7**	91.9	87.6
Hydro./Trich.	100.0	**0.0**	**41.5**	**0.0**	**60.6**	**0.0**	**0.0**	**0.0**	-
EPT/Chiron.	77.1	85.6	90.9	**62.3**	**58.5**	**58.1**	**48.4**	79.3	**0.0**
Shredder/total	**22.0**	85.8	86.3	**59.1**	81.5	84.7	**74.9**	78.0	**52.7**
Scraper/filterers	**18.8**	92.0	80.0	**67.1**	**37.0**	**62.9**	**13.0**	**69.1**	-
No. of habitats	3	2	3	3	2	4	1	4	1

Table 2. Reproducibility (%) (see text for formula) of metrics for duplicate sites from eight reference streams in the three ecoregions for fall 1993. Analysis was done using multihabitat data: cs flow, nonflow and root mat. Reproducibilities < 75% are in bold.

	Prairie		Ozark					Lowland
Metrics	E.F.Crook.	M.Fabius	Burris	Bouef	Ltl.Piney	Huzzah	Marble	Maple Sl.
Taxa	89.4	100.0	100.0	100.0	93.3	95.0	89.7	97.4
Family	95.8	95.7	100.0	95.5	89.3	90.6	93.8	77.3
EPT	88.9	90.9	84.6	86.7	93.3	81.8	85.7	88.9
Biotic Index	98.6	96.7	97.8	96.7	97.0	99.5	95.8	99.1
Shannon Index	97.5	98.5	87.5	99.9	95.7	97.5	97.0	94.1
Simpson Index	94.7	100.0	**59.6**	84.5	**71.7**	76.1	91.3	75.2
Dominant	96.5	94.8	**55.7**	**63.4**	**61.9**	**66.4**	91.5	81.9

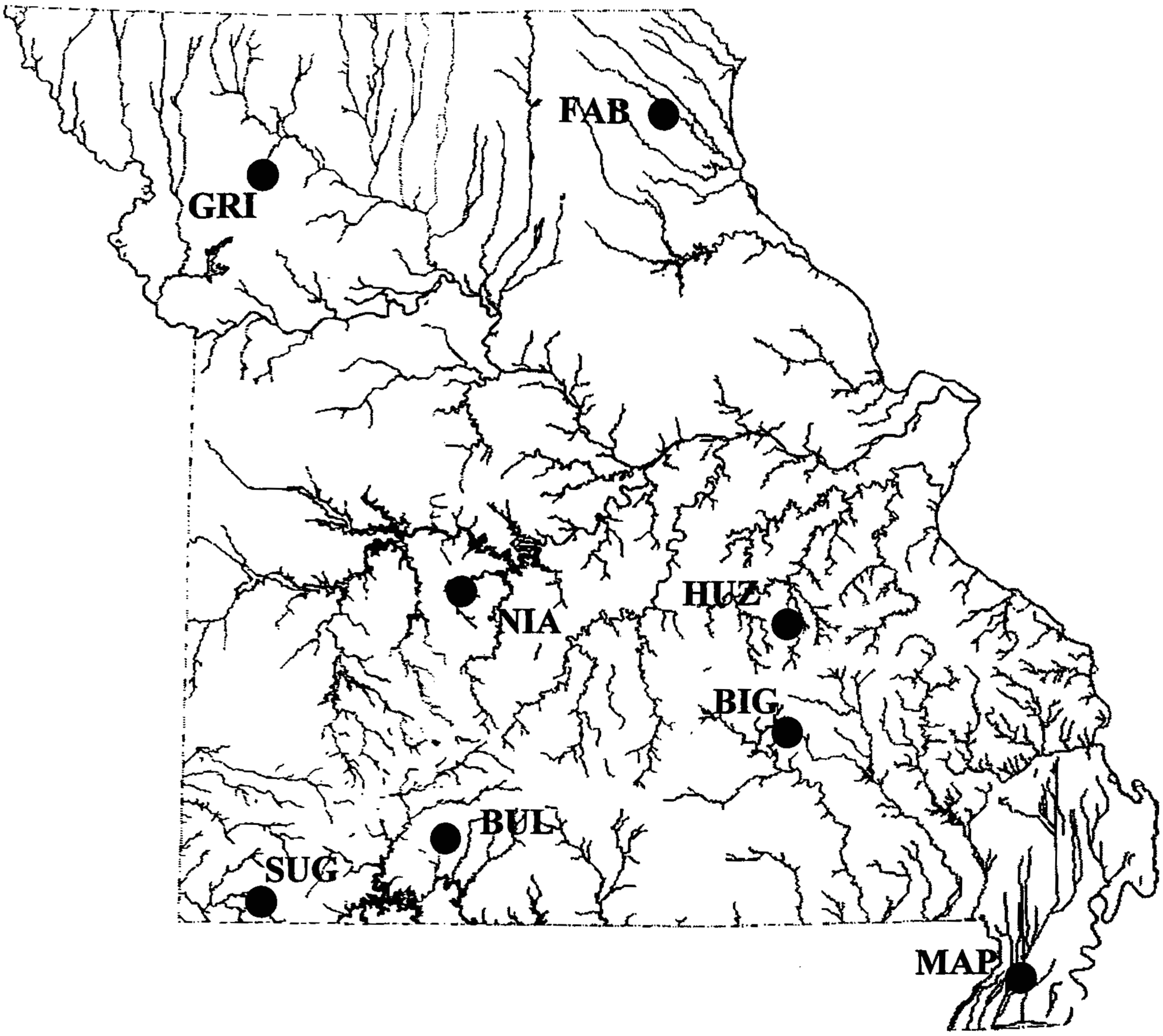


Fig. 1. Location of study streams used to examine the usefulness of replication.

Table 3. Sites and habitats sampled for each site. Site codes on Figure 1-15 are given.

Stream	Code	Region	No. of replicates	cs flow	nonflow	root	fs flow
M. Fabius	FAB	Prairie	4	X	X	X	X
Grindstone	GRI	Prairie	4	X*	X	X	X
Huzzah	HUZ	Ozark	4	X	X	X	
Bull Cr.	BUL	Ozark	4	X	X	X	
Big Sugar	SUG	Ozark	4	X	X	X	
Big Creek	BIG	Ozark	3	X	X	X	
Ltl Niagua	NIA	Ozark	3	X	X	X	
Maple Sl.	MAP	Lowland	4			X	X

* At the second and third replicate sites cs flow was not found, snag was substituted.

each stream were selected and sampled according to our established protocol. We analyzed the data using invertebrates from both individual habitats and from combined multihabitat samples which included cs flow, nonflow, and rootmats. Seven metrics (Total taxa, Family, EPT, BI, Shannon's diversity index, Simpson's diversity index, and % Dominant taxon) were calculated. The changes in mean values of the metrics and the variation (as CV) for cumulative samples were examined to see if metric values remained constant and if variation was substantially reduced by increasing replication.

We first calculated a value for each metric from one sample. We then calculated a value for a second sample, averaged it with the first, and calculated the variation as the CV. The third sample was averaged, and then in most cases a fourth. We regarded decreases of more than 10% as being potentially biologically significant which would have important implications for the interpretation of results.

ANALYSIS BY MULTIHABITAT

Total Taxa (Fig. 2)

Mean values generally remained constant with the addition of samples in both prairie and Ozark streams. When a value did change it decreased just as often as it increased. CVs decreased more than 10% from the second to the last sample in three streams, but the mean values were generally so low in these cases (<20%) that even a 10% change probably does not mean much biologically. The lowland stream showed the most change in both the mean value and in the CV.

EPT

Prairie region streams (FAB, GRI) showed little or no change in mean value with additional sampling. The lowland stream increased its value from 1 to 2.5, which, while probably not biologically meaningful, did decrease the CV from 85 to 52%. Ozark streams showed more variation than prairie streams, with a mean change of 3.8 taxa from the first to the last sample. A change in the CV of greater than 10% occurred at two Ozark streams.

Family

Values remained remarkably constant. The only significant improvement in CV was for the highly variable MAP stream.

Biotic Index

Values changed very little regardless of geographical location. Overall, the average value changed 0.28 from the first to last sample. CVs were extremely low, most less than 10%, and only one, SUG, changed more than 10%.

Shannon Diversity Index

This metric was the most insensitive to increased sampling. The Lowland stream showed anomalous results of an increasing CV. Otherwise CVs were typically less than 10% and index values changed an average of only 0.18 from the first to last sample.

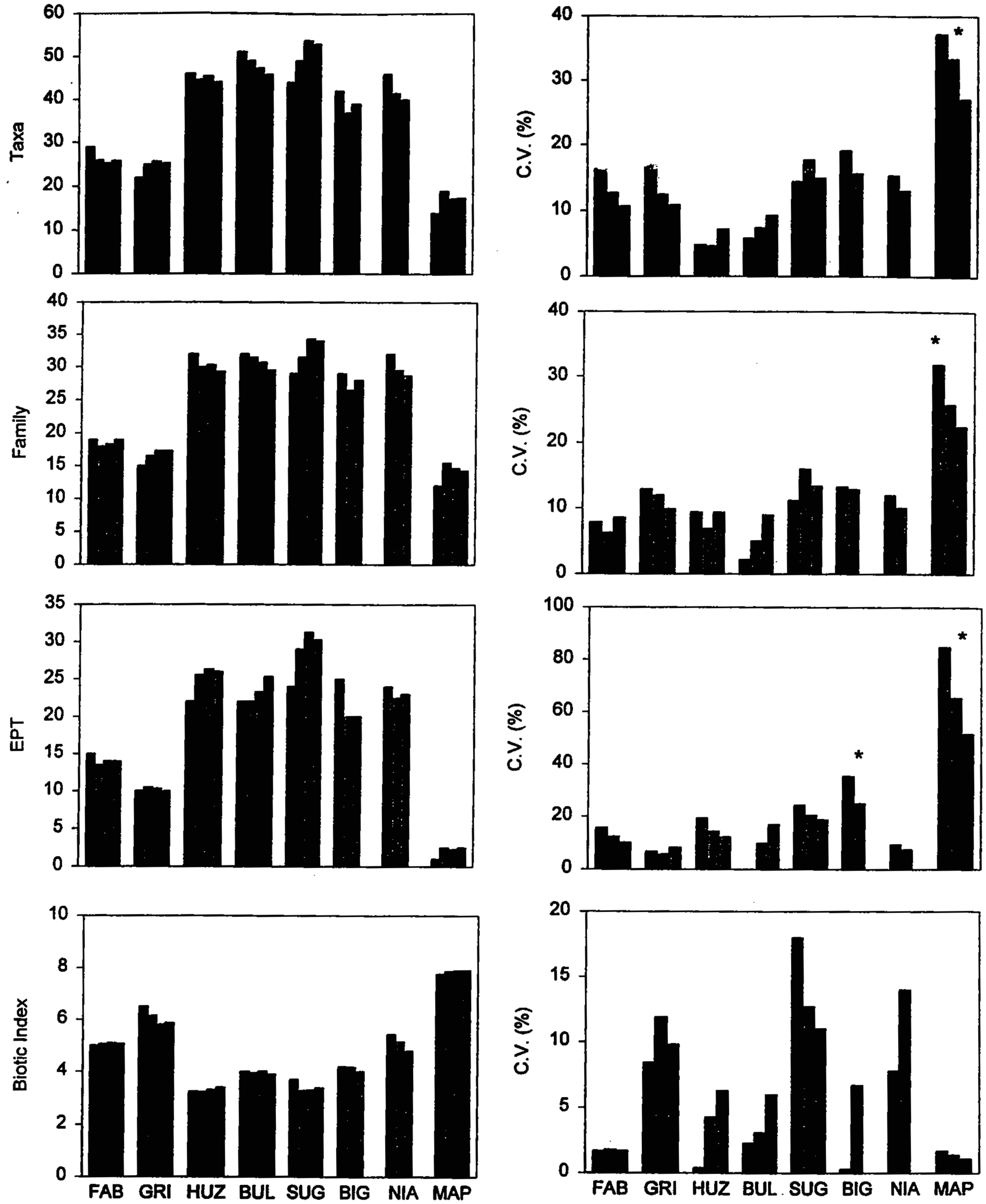


Fig. 2. The effect of sample size on mean values and variation of several biocriteria metrics. Stream abbreviations are given in Table 1. Each set of three or four bars with each stream represents either the mean value of the metric (left figure) or the coefficient of variation (right figure) for consecutive samples; * = a decrease of CV of >10%.

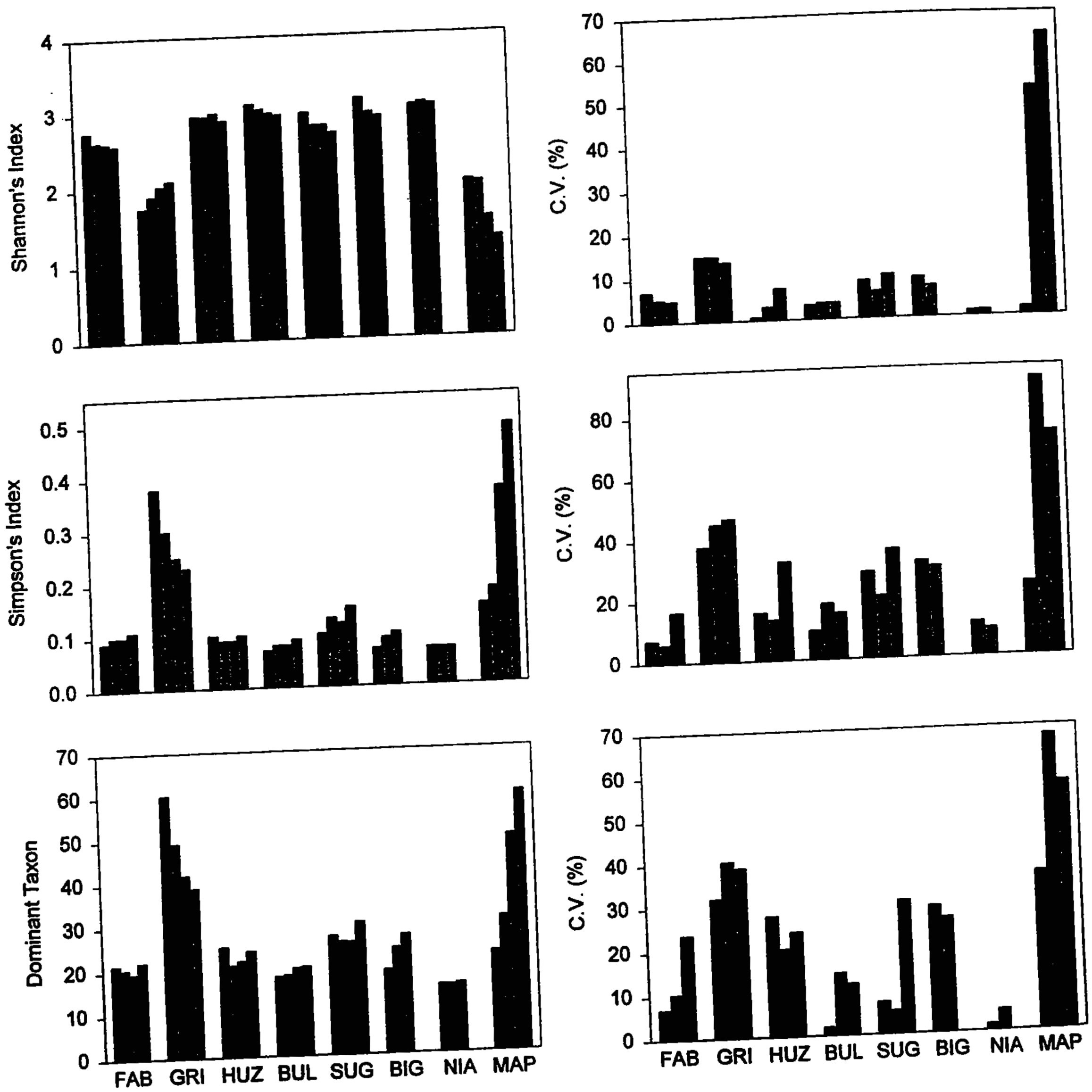


Fig. 2. Continued.

Simpson's Diversity Index

There were no significant improvements in variation for any stream. One prairie stream, GRI, showed consistently lower values with added sampling, while the Lowland stream indicated just the opposite.

% Dominant Taxa

There were no significant improvements in the CVs with added sampling. The pattern of change for the values was almost identical to the Simpson's diversity index.

ANALYSES BY INDIVIDUAL HABITAT

Total Taxa (Fig. 3)

In prairie streams (FAB, GRI) most values within habitats changed little with additional replicates. The greatest range was three taxa for fs flow in one stream and nonflow in the other stream. The CV for the cumulative samples was substantially reduced with addition of replicates in two of the eight stations. Otherwise the CV was essentially the same after two samples as after four. In Ozark streams, numbers of taxa did not change noticeably with additional sampling except in Big Sugar Creek. In only 1 of 15 situations was the CV reduced by more than 10% by the addition of replicates. Values from the Lowland stream were not influenced by additional samples.

EPT Taxa (Fig. 4)

Replicates were comparable in the prairie streams. The range of EPT scores in any one stream was usually 1, with rootmats from one stream having a range of 2.7. In only two of eight situations did the CV improve—i.e., decrease—by about 10% with additional sampling. Consistent results were obtained from Ozark streams. In only 2 of 15 situations was the range within any one stream greater than 4 taxa. In 11 situations the range was 3 or less. In only 2 of 13 trials was the CV reduced more than 10%. Taking additional replicates from the Lowland stream did not reduce the CV.

Biotic Index (Fig. 5)

Mean values for this index were little affected by replication in streams of any region. In prairie and Ozark streams values generally changed no more than 0.5 units from the first to the fourth sample. The lowland stream was little changed.

Shannon Diversity Index (Fig.6)

Additional sampling changed values very little in all streams—generally 0.1-0.2 units in prairie streams and 0.0-0.5 units in Ozark streams. In only one of eight situations in the prairie and 1 of 15 in the Ozarks was the CV reduced by 10% or more. In the Lowland stream, the value was little affected by replication.

These four metrics—Total taxa, EPT, Shannon's diversity index, and the BI were ultimately selected to be incorporated into the final Stream Condition Index. Three other metrics not selected for use in the Stream Condition Index were evaluated—the Simpson's diversity index, % Dominant taxon, and numbers of families. We do not discuss these results in detail, but essentially the same results were shown for both single habitats and multihabitats, and the same conclusions would be drawn if they were going to be used in a final index (Figs. 7-9).

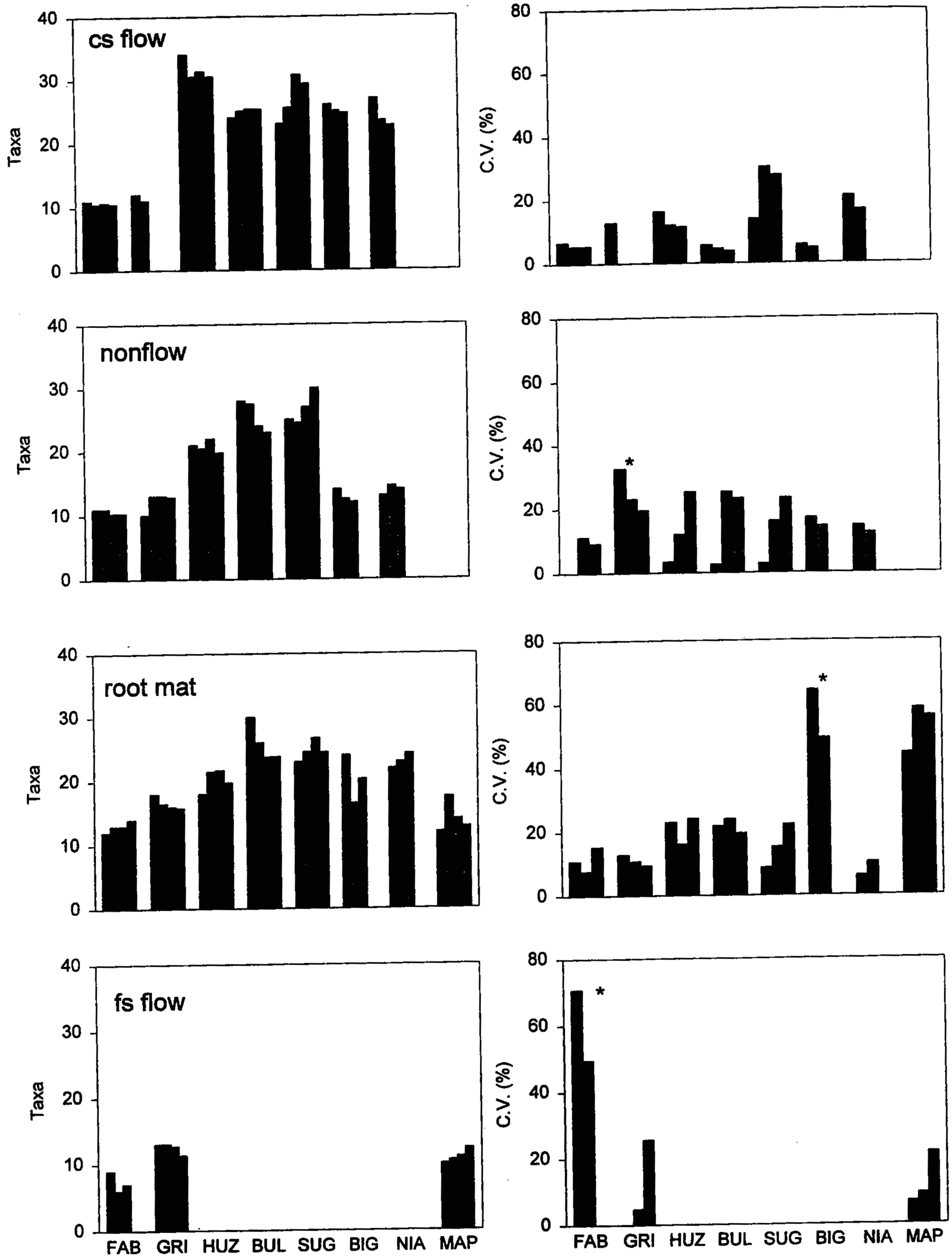


Fig. 3. The effect of sample size on the mean value and coefficient of variation of the Total Taxa metric, for four individual habitats; * = a decrease in CV of >10%. See caption on Fig. 2 for additional figure details.

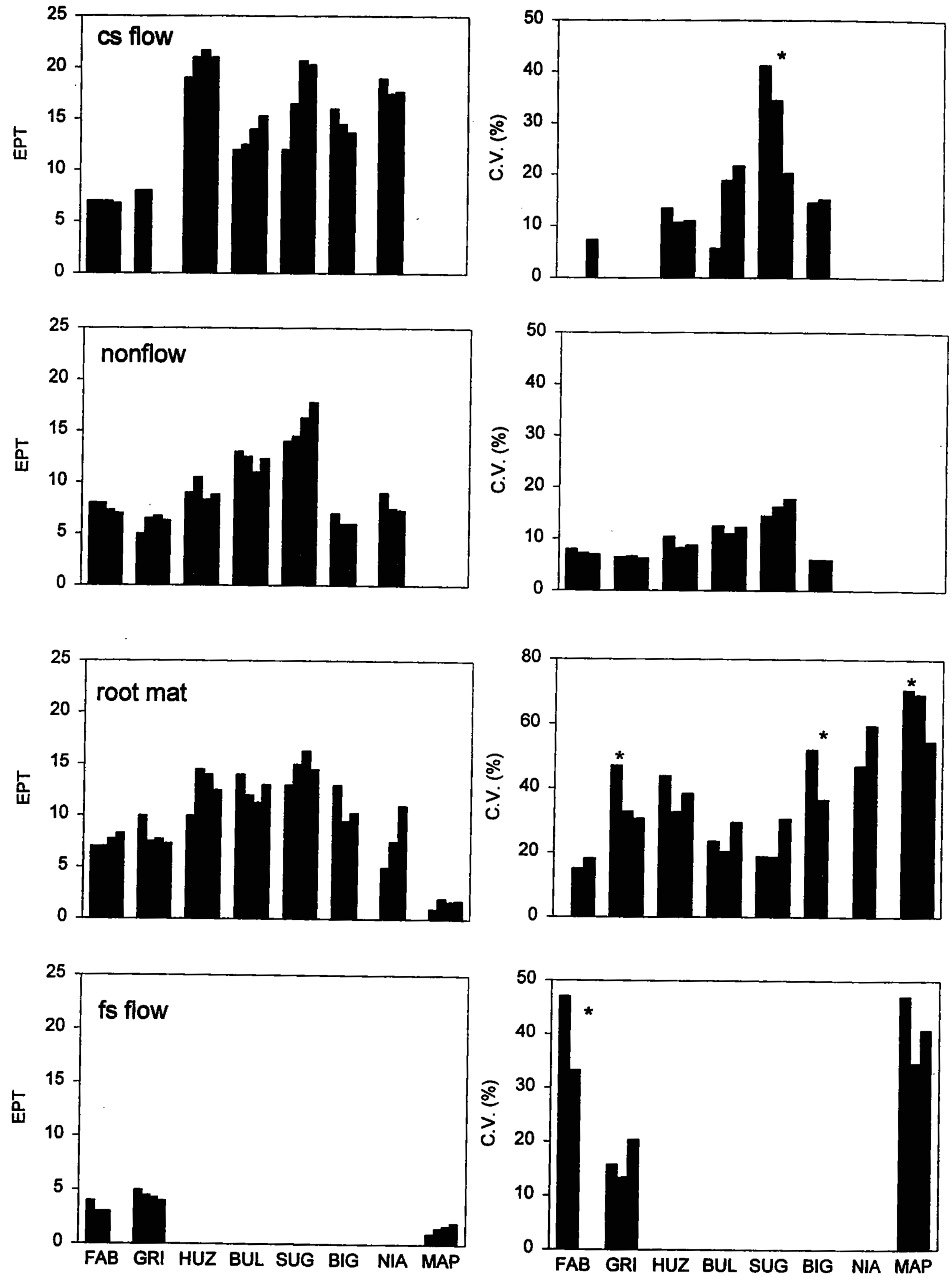


Fig. 4. The effect of sample size on the mean value and coefficient of variation of the EPT metric, for four individual habitats; * = a decrease in CV of >10%. See caption on Fig. 2 for additional figure details.

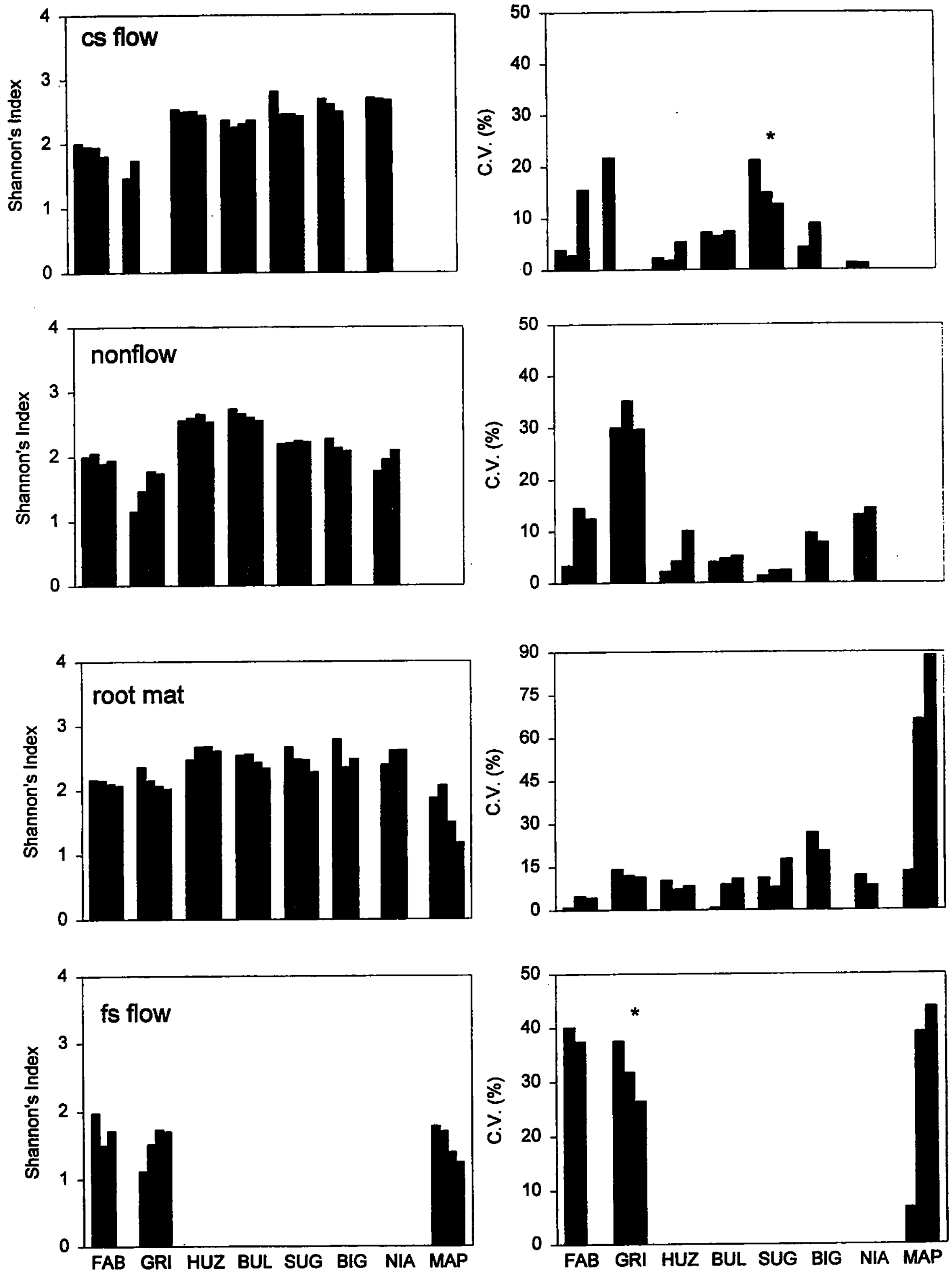


Fig. 5. The effect of sample size on the mean value and coefficient of variation of the Shannon's Index metric, for four individual habitats; * = a decrease in CV of >10%. See caption on Fig. 2 for additional figure details.

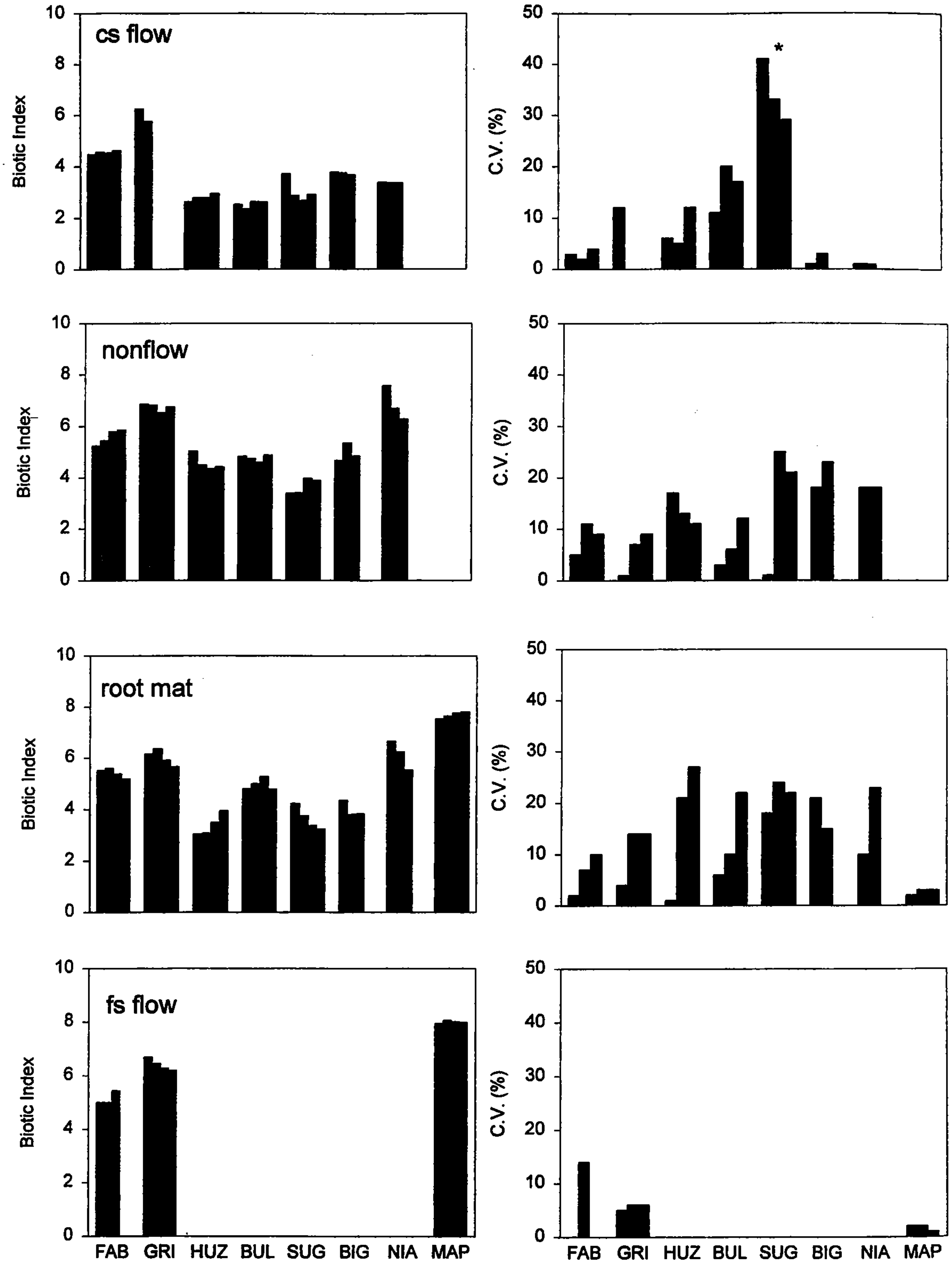


Fig. 6. The effect of sample size on the mean value and coefficient of variation of the biotic index metric, for four individual habitats; * = a decrease in CV of >10%. See caption on Fig. 2 for additional figure details.

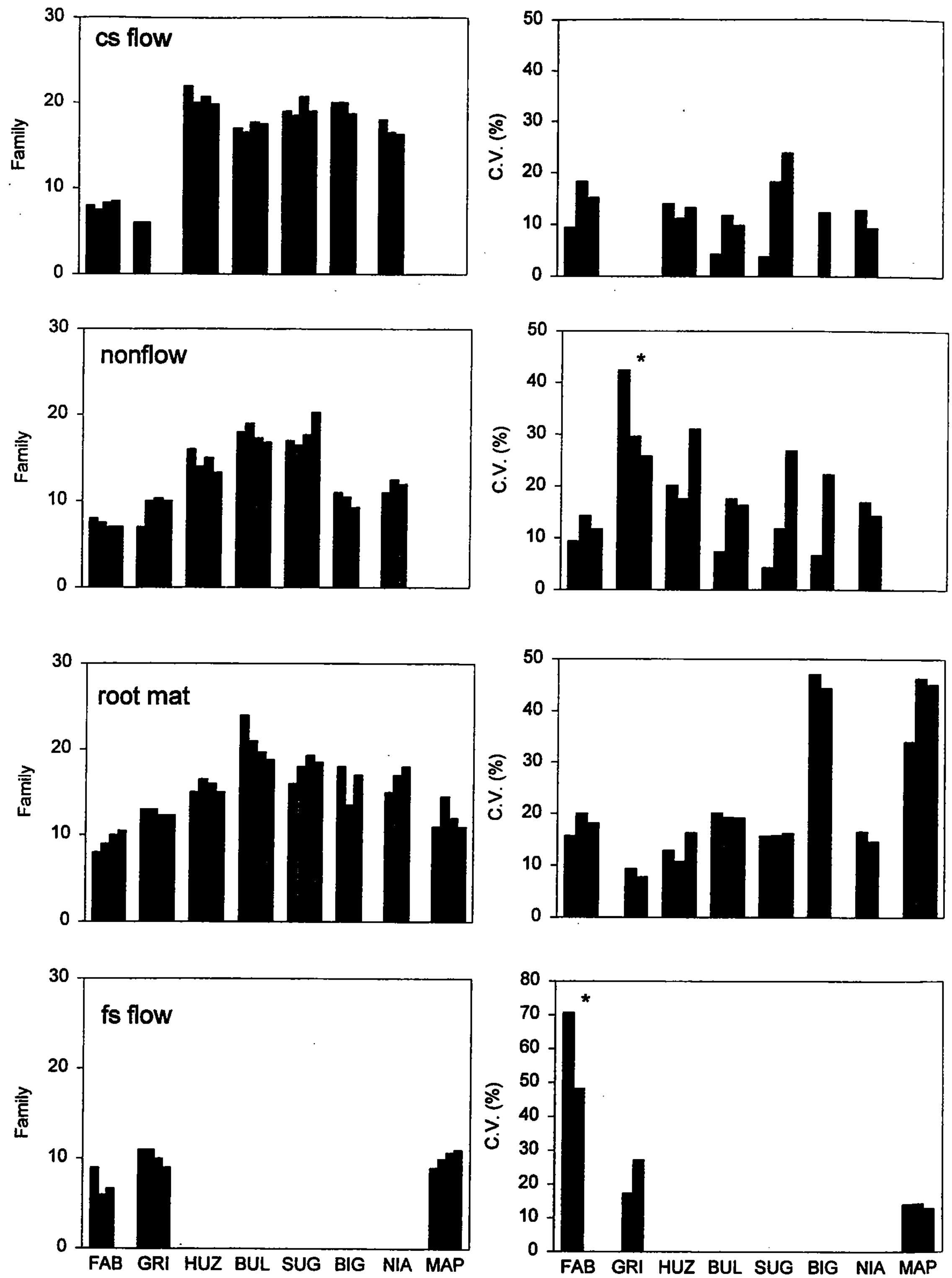


Fig. 7. The effect of sample size on the mean value and coefficient of variation of the Family metric, for four individual habitats; * = a decrease in CV of >10%. See caption on Fig. 2 for additional figure details.

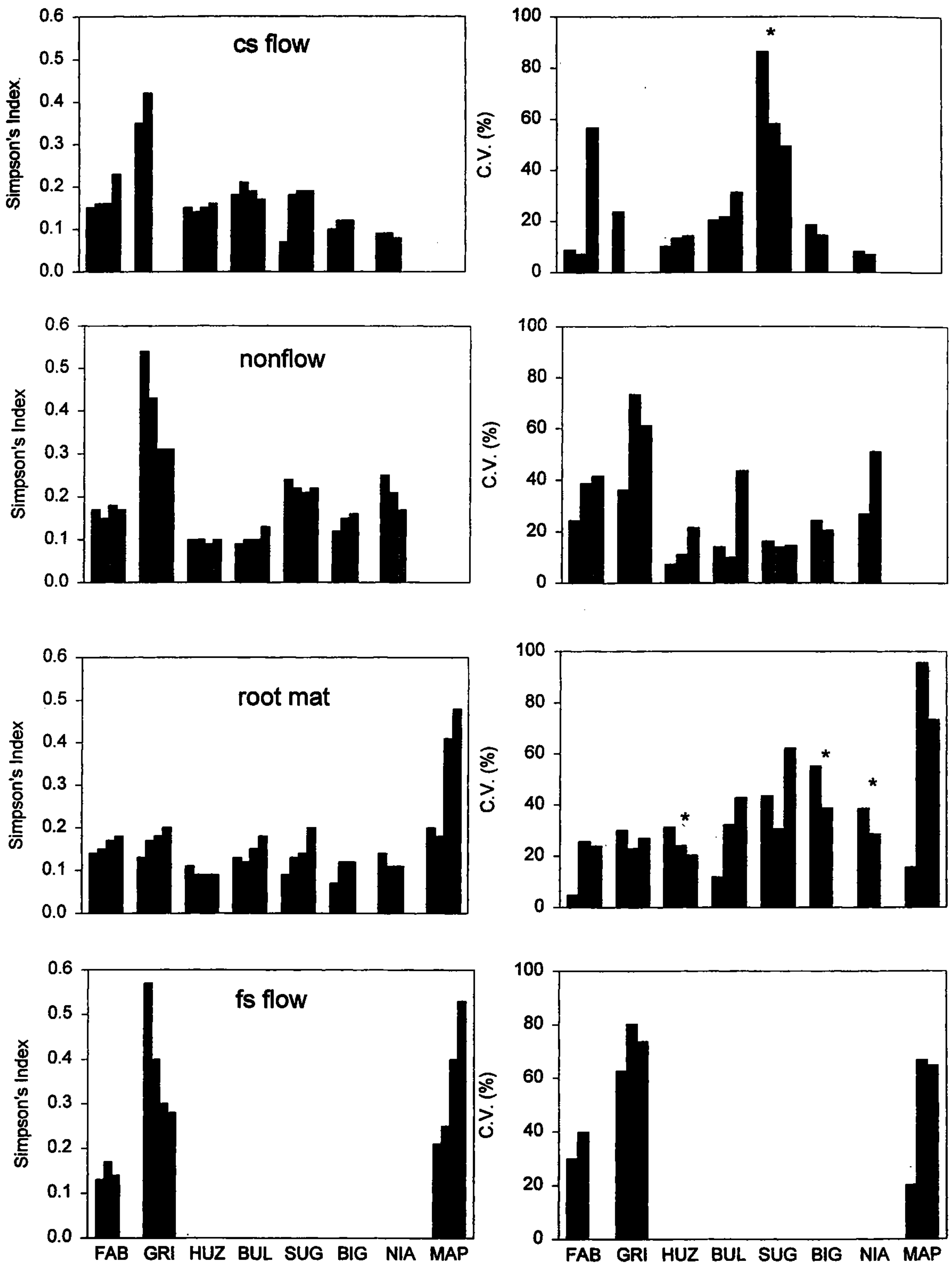


Fig. 8. The effect of sample size on the mean value and coefficient of variation of the Simpson's index metric, for four individual habitats; * = a decrease in CV of >10%. See caption on Fig. 2 for additional figure details.

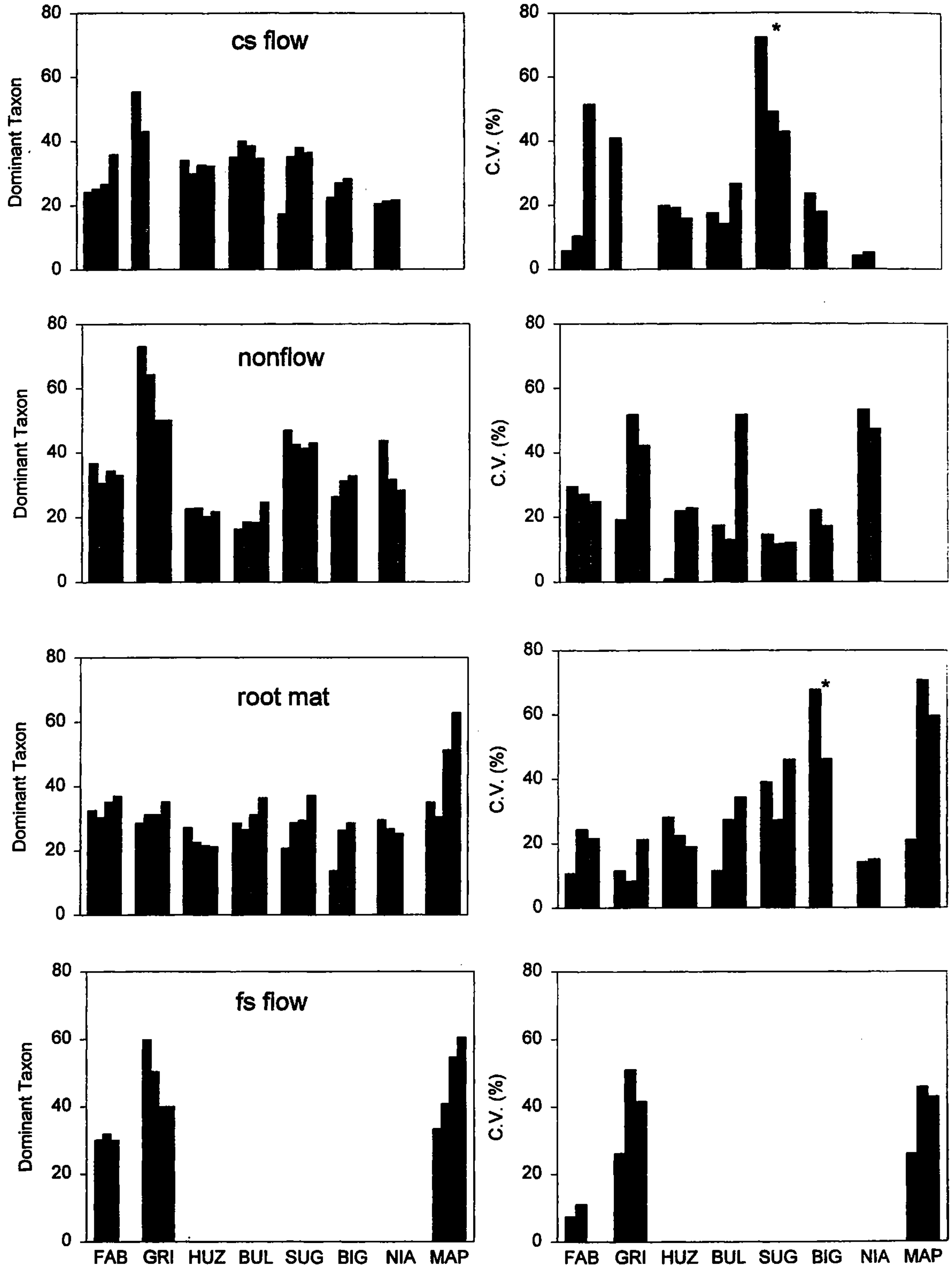


Fig. 9. The effect of sample size on the mean value and of the % dominant metric, for four individual habitats; * = a decrease in CV of >10%. See caption on Fig. 2 for additional figure details.

EVALUATION OF MEASUREMENT ERROR

Most biocriteria are developed so that a site score from a single stream reach can be compared against a single or mean reference scores to determine its placement in categories of impairment. However, we must also account for variance associated with the measurement of that test site. To do so, replication is required and we used data from replicated sites in spring of 1994: two streams in the prairie region and five streams in the Ozark region to evaluate measurement error. We examined the minimum detectable difference--i.e., how different a test stream metric must be from the reference mean value, when the number of reaches sampled was one, two, or three.

The change needed in an individual metric for it to be considered significantly different can be calculated by using a rearrangement of the t-test formula (Parkinson et al. 1988).

$$p^2 = ((CV)^2 \, k/N)$$

where N is the number of samples (i.e., reaches), in this case either one, two, or three; k is a constant that varies with alpha and statistical power (Snedecor and Cochran 1967). CV is the coefficient of variation (sd/mean of metric values), and p is the change expressed as a proportion of the mean. We used alpha = 0.05 and 80% statistical power which gave a k = 12.57, and assumed a one-tail test.

Table 4 gives the approximate error associated with both a one- and two-sample comparison to a reference situation for four different metrics. For example, Total taxa from a test stream in the Ozark region must be 17 fewer taxa using data from one reach, 12 fewer taxa using two reaches, or 10 fewer using three reaches to be considered statistically different (or degraded). For all metrics in both regions the increase in precision by addition of the second sample is moderate, and sampling more than two reaches may not be worth the resources.

CONCLUSIONS

Formal statistical tests of our sampling adequacy were probably not appropriate (Norris et al. 1992), and probably not necessary. We were not able to locate comparable studies evaluating replicate sites, only studies evaluating total numbers of individual samples within a site (e.g., Stark 1993). However, we were encouraged by the within-site reproducibility and the stability of metric values as sampling increased. We conclude that our sampling within a site is completely adequate, and replicating reaches within a stream is usually not necessary. Sampling one location appears sufficient, whereas two would be optimum. Taking any more than two samples would not be warranted.

Table 4. Statistics used to determine the detectable difference for each of four metrics at α=0.05 and a power of 80% (Parkinson et al. 1988). Data from spring 1994, Ozarks. X = mean value of each metric.

Metric	$\bar{x}$	CV (%)	Significant difference using:		
			1 sample reach	2 sample reaches	3 sample reaches
Ozarks (N = 5)					
Total taxa	44	11	17	12	10
EPT	25	16	14	10	8
Biotic Index	3.9	8	1.0	0.77	0.63
Shannon's index	2.90	6	0.60	0.43	0.35
Prairie (N = 2)					
Total taxa	26	10	9	6	5
EPT	12	9	4	3	2
Biotic Index	5.4	5	0.94	0.66	0.54
Shannon's index	2.30	9	0.72	0.51	0.41

Chapter 12

TEMPORAL VARIATION OF THE INVERTEBRATE FAUNA

INTRODUCTION

The benthic invertebrate community at any particular site in a Missouri stream consists of perhaps hundreds of species from a wide variety of taxonomic orders. Many have unique life history strategies and life cycles relating to rates of mortality and individual growth, immigration and emigration, and periods of time spent in egg or adult stage. Each taxon pursuing its own natural cycles of abundance potentially results in an ever changing aggregate of populations—and of community structure. Thus, metrics or an index derived at two different times could well reflect natural variation, and complicate the determination of impairment.

This chapter evaluates the magnitude of community change both seasonally and between years by comparing commonly used metrics. The question is important in deciding how often reference streams need to be sampled. Do we have to sample REF streams each season, or every year, or can a typical REF condition be established once and thereafter used to compare with test conditions? Certainly seasonal and between year differences in community structure exist, but do these differences alter metric values? Comparisons were made using the identical locations, first between two seasons of a year, then between the same season of different years. Comparisons were statistically tested using a paired t-test for each metric. Finally, trends over time were examined using box plots.

TEMPORAL COMPARISONS

A comparison of metrics between seasons (spring 1993 and fall 1993)

Only identical habitats were used. For the Prairie region, three comparisons were made: multihabitat consisting of cs flow and rootmats (eight sites each season; Table 1) and single habitat comparisons of cs flow (eight sites/season; Table 2) and rootmats (14 sites/season; Table 3). For Ozark region comparisons a multihabitat analysis using cs flow, nonflow, and rootmats (15 sites/season; Table 1) and a single habitat analysis of cs flow (26 sites/season; Table 2) were made.

Multihabitat Results

For Ozark streams, no significant differences were found $P > 0.10$. For prairie stream comparisons there were no significant differences (paired t-test, $P >$ 0.05) for any individual metric between seasons (Table 1). The EPT and BI were marginally significant ($P < 0.10$).

Single Habitat Results

Invertebrates from the cs flow habitat were examined for Ozark streams (Table 2). Seasonal values were very comparable. The only metric showing even marginal significance was Shannon's diversity index ($P = 0.062$). For the Prairie region, cs flow results were the same with only a single metric, EPT, showing marginal

Table 1. Differences in metrics of reference streams between spring and fall 1993, multi-habitat.

	Prairie (cs flow + root mats)													
	Taxa		Family		EPT		Biotic Index		Shannon		Simpson		Dominant	
Streams	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall
Honey	20	29	12	16	5	11	6.0	5.8	2.19	2.20	0.17	0.19	25.4	37.4
E. Fk. Grand	26	26	23	18	5	11	6.9	3.6	2.51	1.87	0.14	0.30	29.6	52.3
Grindstone	31	31	20	20	11	10	6.3	5.7	2.95	2.43	0.07	0.17	12.1	37.7
W. Locust	34	25	16	14	7	11	6.8	5.8	2.36	1.61	0.18	0.37	37.8	58.2
Petite Saline	25	27	17	18	5	5	6.7	6.5	1.77	2.61	0.28	0.11	46.3	19.7
Mid. Fabius	30	23	22	17	9	8	5.9	5.8	2.58	2.47	0.13	0.12	28.1	27.0
Loutre	30	28	21	15	3	7	6.6	6.1	2.64	2.28	0.12	0.20	28.8	40.0
North R.	29	33	23	18	11	11	6.5	6.1	2.80	2.30	0.11	0.16	26.6	25.2
Means	28.1	27.8	19.3	17.0	7.0	9.3	6.5	5.7	2.48	2.22	0.15	0.20	29.3	37.2
SD	4.3	3.2	3.9	1.9	3.0	2.3	0.4	0.9	0.37	0.33	0.06	0.09	9.9	13.3
Paired t-test		0.860		0.122		0.076		0.077		0.198		0.204		0.238

	Ozark (cs flow + nonflow + root mats)													
	Taxa		Family		EPT		Biotic		Shannon		Simpson		Dominant	
	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall
R. Aux Vasse	61	51	31	23	19	15	6.3	6.1	3.20	3.13	0.09	0.08	25.7	18.7
Apple	44	60	25	34	11	15	6.5	5.9	2.90	2.93	0.09	0.11	19.6	24.7
Saline	53	52	27	33	19	18	6.3	5.8	2.64	3.03	0.15	0.09	33.6	17.9
Bouef	38	42	24	21	7	15	6.6	6.0	2.33	2.91	0.20	0.10	41.4	24.3
Cedar	47	51	26	27	18	11	5.9	6.7	3.16	3.21	0.07	0.06	15.9	14.3
Pom. de Terre	53	53	33	34	14	16	6.2	6.6	3.19	2.96	0.07	0.10	16.6	23.7
Deer Cr.	53	54	32	34	16	14	5.6	5.8	3.16	3.45	0.07	0.04	17.0	8.9
Ltl. Niangua	57	45	30	29	16	15	6.7	5.1	3.06	3.10	0.09	0.07	19.4	16.2
Bull	48	49	25	25	18	14	5.1	4.5	3.22	2.95	0.06	0.10	15.7	22.4
Spring (Doug.	60	56	28	27	16	14	5.2	4.3	3.44	3.01	0.05	0.10	12.9	25.1
Sinking (Shan	47	52	23	30	14	19	4.4	4.3	3.26	2.34	0.05	0.27	12.1	50.7
Ltl. Black	56	62	31	31	16	13	5.4	5.5	3.11	3.31	0.09	0.06	24.9	15.4
Ltl. Piney	51	45	33	28	22	14	5.9	4.7	2.49	2.88	0.22	0.10	45.0	23.6
Meramec	61	65	33	33	21	19	5.0	5.6	3.17	3.31	0.09	0.05	24.9	11.7
Huzzah	47	57	27	29	22	18	3.8	5.4	2.70	3.26	0.12	0.07	21.6	22.5
Means	51.7	52.9	28.5	29.2	16.6	15.3	5.7	5.5	3.00	3.05	0.10	0.09	23.1	21.3
SD	6.7	6.4	3.5	4.1	4.1	2.3	0.8	0.8	0.32	0.26	0.05	0.05	9.6	9.3
Paired t-test		0.534		0.565		0.282		0.371		0.626		0.656		0.657

Table 2. Differences in metrics of reference streams between spring and fall 1993, cs flow.

Streams	Taxa		Family		EPT		Biotic Index		Shannon		Simpson		Dominant	
	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall
Prairie Region														
Honey	13	19	7	10	5	9	5.8	5.9	2.08	1.58	0.16	0.37	23.2	58.7
E. Fk. Grand	10	12	7	8	5	9	5.8	3.2	1.58	1.28	0.26	0.46	36.9	65.8
Grindstone	18	12	11	9	8	7	6.2	5.7	2.58	1.75	0.09	0.30	15.1	51.4
W. Locust	14	15	6	9	3	8	6.5	5.7	1.48	1.20	0.39	0.50	59.6	68.4
Petite Saline	14	17	10	10	3	5	6.7	6.3	1.69	2.25	0.29	0.16	48.9	33.7
Mid. Fabius	20	10	15	8	7	4	5.6	6.0	2.18	1.90	0.19	0.21	36.4	39.6
Loutre	19	20	11	9	3	6	6.4	6.3	2.22	2.14	0.18	0.21	37.3	39.2
North R.	18	28	11	16	5	10	5.7	6.0	2.02	2.21	0.25	0.18	46.8	29.7
MEANS	15.8	16.6	9.8	9.9	4.9	7.3	6.1	5.6	1.98	1.79	0.23	0.30	38.0	48.3
SD	3.5	5.8	3.0	2.6	1.9	2.1	0.4	1.0	0.37	0.41	0.09	0.13	14.2	14.9
Paired t-test		0.708		0.928		0.055		0.248		0.245		0.169		0.215
Ozark Region														
R.Aux Vas	28	16	17	10	11	10	4.5	5.4	2.74	2.03	0.10	0.19	20.9	30.0
Apple	18	29	12	21	7	9	5.1	5.6	2.06	2.30	0.20	0.16	35.1	26.9
Saline	26	32	13	20	13	12	5.0	5.3	2.36	2.54	0.14	0.13	23.6	25.5
Ltl. Whitew	22	28	13	15	10	11	5.5	5.7	2.49	2.31	0.13	0.16	26.1	32.5
Burris	16	25	10	14	7	8	6.2	5.3	1.89	2.39	0.24	0.13	41.8	18.2
Bouef	15	20	7	13	4	8	6.1	5.6	1.64	2.61	0.34	0.09	54.3	17.7
Cedar	28	29	16	16	15	9	5.5	5.8	2.78	2.57	0.10	0.12	21.9	25.7
Pomme	25	26	16	20	13	13	5.8	5.3	2.77	2.50	0.09	0.17	20.4	37.9
Deer Cr.	23	26	16	19	8	10	5.1	5.1	2.64	2.92	0.11	0.07	22.9	13.2
Ltl. Niangua	28	18	18	13	10	9	6.0	4.4	2.46	2.29	0.16	0.14	33.8	29.1
Ltl. Maries	28	27	22	16	12	11	4.9	5.7	2.80	2.34	0.09	0.17	16.8	33.3
Big Sugar	32	20	18	15	16	12	4.6	3.1	2.97	2.14	0.08	0.23	18.3	45.5
Bull	23	25	12	14	12	11	4.3	3.5	2.69	2.41	0.10	0.17	20.5	34.3
Spring (Doug.)	20	23	13	16	6	9	4.5	3.1	2.62	2.19	0.09	0.20	17.6	38.9
North Fork	25	22	13	17	10	12	3.7	3.4	2.65	2.43	0.12	0.13	26.3	27.1
Jacks Fork	28	17	18	14	11	6	4.1	3.3	2.82	2.08	0.08	0.18	16.2	32.0
Sinking (Shan.	21	17	12	12	7	5	3.9	3.5	2.70	1.33	0.08	0.50	12.4	69.8
Big Cr.	28	30	13	20	10	13	4.0	4.0	2.72	2.81	0.12	0.09	31.1	16.8
Ltl. Black	18	26	12	18	9	9	4.4	4.6	2.09	2.45	0.24	0.14	47.5	33.0
W. Piney	34	17	27	15	15	9	4.7	3.0	3.14	1.87	0.06	0.29	15.3	52.3
Ltl. Piney	26	26	20	18	12	12	5.4	4.4	2.40	2.50	0.15	0.13	28.3	26.5
Meramec	36	35	20	21	16	14	5.0	5.3	3.03	2.95	0.08	0.07	18.7	16.0
Huzzah	31	30	21	16	15	12	3.9	5.2	2.53	2.86	0.15	0.07	28.9	11.3
Marble	36	27	25	14	15	11	4.9	5.2	3.17	2.60	0.06	0.13	14.8	30.9
E. Fk. Black	32	30	22	22	15	14	4.4	4.7	3.17	2.96	0.05	0.07	10.2	17.5
Sinking (Reyn.)	27	25	17	17	12	11	4.2	3.8	3.02	2.67	0.06	0.11	11.2	27.9
MEANS	25.9	24.8	16.3	16.4	11.2	10.4	4.8	4.6	2.63	2.42	0.12	0.16	24.4	29.6
SD	5.8	5.1	4.8	3.1	3.3	2.2	0.7	1.0	0.39	0.36	0.07	0.09	11.0	12.7
Paired t-test		0.449		0.916		0.142		0.134		0.062		0.182		0.192

Table 3. Differences in metrics of reference streams between spring and fall 1993, root mats, Prairie region.

Streams	Taxa		Family		EPT		Biotic Index		Shannon		Simpson		Dominant	
	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall
White Cloud	19	19	14	10	2	5	7.4	6.1	2.00	1.87	0.24	0.32	43.4	54.9
Long Br. Platt	10	23	6	12	2	7	7.9	7.1	0.86	2.57	0.64	0.10	78.8	16.2
Honey	16	21	10	13	5	8	6.2	5.7	1.97	2.36	0.21	0.14	31.8	27.5
E. Fk. Grand	24	22	12	13	4	9	7.1	5.2	2.43	2.27	0.16	0.17	33.3	34.5
Grindstone	26	26	18	17	10	8	6.5	5.8	2.79	2.70	0.09	0.11	22.2	26.2
W. Fk. Big	21	20	9	14	8	8	6.9	5.4	2.37	2.33	0.17	0.15	36.8	30.8
No Cr.	20	20	14	13	7	3	7.1	6.5	2.21	2.32	0.18	0.16	36.1	33.0
W. Locust	28	17	14	10	7	7	7.4	6.1	2.55	1.82	0.13	0.27	27.5	45.0
Spring (Adair	21	17	14	12	8	9	7.4	4.7	2.18	2.37	0.20	0.12	36.8	18.8
E. Fk. Crooke	24	21	17	13	7	3	6.7	7.0	2.71	2.46	0.10	0.13	24.8	27.5
Mid. Fabius	23	17	17	13	8	5	6.6	5.4	2.59	2.17	0.12	0.18	29.0	34.2
North R.	29	17	19	9	7	6	7.4	6.1	2.81	2.07	0.09	0.19	20.0	34.2
Petite Saline	19	20	16	15	3	4	7.3	6.7	1.97	2.25	0.26	0.16	48.2	29.8
Loutre	18	18	16	13	1	5	7.3	6.1	2.03	2.02	0.20	0.22	33.1	40.2
MEAN	21.3	19.9	14.0	12.6	5.6	6.2	7.1	6.0	2.25	2.25	0.20	0.17	35.8	32.3
SD	5.0	2.7	3.7	2.1	2.8	2.1	0.5	0.7	0.50	0.25	0.14	0.06	14.6	9.9
Paired t-test		0.403		0.244		0.507		0.000		0.966		0.539		0.525

significance ($P = 0.055$). Comparison of prairie rootmats also showed similar values between seasons of the same year. The only significant difference was found for the BI ($p < 0.01$; Table 3).

Results so far indicate few significant differences between seasons. The chances of finding significant differences between two datasets depends upon the variation within each dataset: the smaller the variation, the more likely there are to be differences. We consider the variation within each of our metrics for the REF stream sites to be remarkably small, and were surprised that more significant differences did not exist. Even so, we attempted to "push" this idea by making comparisons between datasets possessing even less variation. We streamlined our datasets into prairie-upper by removing four sites, and Ozark-central by removing 12 sites (Table 4) so the communities would be even more similar.

Results did not change for the prairie-rootmat comparison which produced identical results to the full prairie dataset (Tables 3 and 4). For prairie cs flow habitat comparison, three metrics: Shannon's diversity index, Simpson's diversity index, and % Dominant taxon were significantly different (Table 4), whereas only EPT was marginally different with the full prairie dataset (Table 2). For the modified Ozark dataset (Table 4), all the metrics except family were significantly different at least at the $P = 0.10$ level.

A Comparison of Metrics Between Seasons (spring and fall 1994)

Five Ozark REF streams were compared between spring and fall 1994 (Table 5). The means of two replicates at each stream were used for comparison using multihabitat (cs flow + nonflow + rootmats) and single habitat cs flow. Because midges of the family Chironomidae were not identified to genera in the spring 1994 study, this group was omitted from the analysis entirely. For the multihabitat analysis (Table 5) the metrics EPT and Shannon's diversity Index were significantly different at $P < 0.05$. Family was different at the $P < 0.10$. Using the single habitat (cs flow) dataset (Table 6), tests showed only the BI was significantly different between seasons ($P < 0.05$). Three other metrics were seasonally different at the $P = 0.10$ level of significance.

These results from 1994 suggest some seasonal differences existed for some metrics. The metrics most sensitive to seasonal changes appear to be EPT, BI, and Shannon's diversity index.

A Comparison of Metrics Between Years (fall 1993 and fall 1994)

Five Ozark REF streams sampled in the fall of 1993 and the fall of 1994 were used to examine year to year changes. Metrics were compared using both multihabitat data (cs flow, nonflow, and rootmats) and by a single habitat (cs flow). For the 1994 data, means from replicate sites were used, while the 1993 data was from a single site.

For multihabitat data, comparisons of Total taxa, Family, and EPT metrics were found to be significantly different between years ($P < 0.05$; Table 7). For cs flow comparisons there was no significant year–to-year difference for any of the metrics (Table 8).

A Comparison of Metrics Using Box and Whisker Plots

Box and whisker plots from data collected at REF sites in the Ozark region in different seasons or years were used to further examine the temporal differences for our four core metrics. For this analysis we added the summer 1995 dataset. For

Table 4. Differences in metrics of reference streams between spring and fall 1993.

Streams	Taxa		Family		EPT		Biotic Index		Shannon		Simpson		Dominant	
	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall
							Prairie-Upper, cs flow							
Honey	13	19	7	10	5	9	5.8	5.9	2.08	1.58	0.16	0.37	23.2	58.7
E. Fk. Grand	10	12	7	8	5	9	5.8	3.2	1.58	1.28	0.26	0.46	36.9	65.8
Grindstone	18	12	11	9	8	7	6.2	5.7	2.58	1.75	0.09	0.30	15.1	51.4
W. Locust	14	15	6	9	3	8	6.5	5.7	1.48	1.20	0.39	0.50	59.6	68.4
MEANS	13.8	14.5	7.8	9.0	5.3	8.3	6.1	5.1	1.93	1.45	0.23	0.40	33.7	61.1
SD	3.3	3.3	2.2	0.8	2.1	1.0	0.4	1.3	0.51	0.26	0.13	0.09	19.5	7.6
Paired t-test		0.783		0.368		0.114		0.216		0.032		0.005		0.024
							Prairie-Upper, root mats							
White Cloud	19	19	14	10	2	5	7.4	6.1	2.00	1.87	0.24	0.32	43.4	54.9
Long Br. Platte	10	23	6	12	2	7	7.9	7.1	0.86	2.57	0.64	0.10	78.8	16.2
Honey	16	21	10	13	5	8	6.2	5.7	1.97	2.36	0.21	0.14	31.8	27.5
E. Fk. Grand	24	22	12	13	4	9	7.1	5.2	2.43	2.27	0.16	0.17	33.3	34.5
Grindstone	26	26	18	17	10	8	6.5	5.8	2.79	2.70	0.09	0.11	22.2	26.2
W. Fk. Big	21	20	9	14	8	8	6.9	5.4	2.37	2.33	0.17	0.15	36.8	30.8
No Cr.	20	20	14	13	7	3	7.1	6.5	2.21	2.32	0.18	0.16	36.1	33.0
W. Locust	28	17	14	10	7	7	7.4	6.1	2.55	1.82	0.13	0.27	27.5	45.0
Spring (Adair)	21	17	14	12	8	9	7.4	4.7	2.18	2.37	0.20	0.12	36.8	18.8
E. Fk. Crooked	24	21	17	13	7	3	6.7	7.0	2.71	2.46	0.10	0.13	24.8	27.5
MEAN	20.9	20.6	12.8	12.7	6.0	6.7	7.1	6.0	2.21	2.31	0.21	0.17	37.2	31.4
SD	5.2	2.7	3.6	2.0	2.7	2.3	0.5	0.8	0.55	0.28	0.16	0.07	15.9	11.5
Paired t-test		0.881		0.934		0.523		0.002		0.639		0.471		0.437
							Ozark-Central, cs flow							
Big Sugar	32	20	18	15	16	12	4.6	3.1	2.97	2.14	0.08	0.23	18.3	45.5
Bull	23	25	12	14	12	11	4.3	3.5	2.69	2.41	0.10	0.17	20.5	34.3
Spring (Doug.)	20	23	13	16	6	9	4.5	3.1	2.62	2.19	0.09	0.20	17.6	38.9
North Fork	25	22	13	17	10	12	3.7	3.4	2.65	2.43	0.12	0.13	26.3	27.1
Jacks Fork	28	17	18	14	11	6	4.1	3.3	2.82	2.08	0.08	0.18	16.2	32.0
Sinking (Shan.	21	17	12	12	7	5	3.9	3.5	2.70	1.33	0.08	0.50	12.4	69.8
Big Cr.	28	30	13	20	10	13	4.0	4.0	2.72	2.81	0.12	0.09	31.1	16.8
W. Piney	34	17	27	15	15	9	4.7	3.0	3.14	1.87	0.06	0.29	15.3	52.3
Ltl. Piney	26	26	20	18	12	12	5.4	4.4	2.40	2.50	0.15	0.13	28.3	26.5
Meramec	36	35	20	21	16	14	5.0	5.3	3.03	2.95	0.08	0.07	18.7	16.0
Huzzah	31	30	21	16	15	12	3.9	5.2	2.53	2.86	0.15	0.07	28.9	11.3
Marble	36	27	25	14	15	11	4.9	5.2	3.17	2.60	0.06	0.13	14.8	30.9
E. Fk. Black	32	30	22	22	15	14	4.4	4.7	3.17	2.96	0.05	0.07	10.2	17.5
Sinking (Reyn.)	27	25	17	17	12	11	4.2	3.8	3.02	2.67	0.06	0.11	11.2	27.9
MEANS	28.5	24.6	17.9	16.5	12.3	10.8	4.4	4.0	2.83	2.41	0.09	0.17	19.3	31.9
SD	5.2	5.6	4.9	2.9	3.2	2.7	0.5	0.8	0.25	0.46	0.03	0.11	6.8	15.9
Paired t-test		0.030		0.334		0.068		0.074		0.008		0.036		0.034

Table 5. Differences in metrics between spring and fall 1994, Ozark, multihabitat, without chironomids

Streams	Taxa		Family		EPT		Biotic Index		Shannon		Simpson		Dominant	
	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall
Huzzah Cr.	44.5	51.5	30.0	36.0	25.5	24.0	3.3	4.0	2.93	2.72	0.09	0.12	21.0	24.0
Big Cr.	37.0	46.0	26.5	32.0	20.0	18.0	4.2	4.6	2.96	2.67	0.09	0.15	24.4	33.0
Bull Cr.	49.0	42.5	31.5	31.0	22.0	18.5	4.0	3.7	3.03	2.66	0.08	0.17	18.5	38.2
Big Sugar Cr.	49.0	49.0	31.5	32.0	29.0	22.5	3.3	3.9	2.80	2.78	0.13	0.12	25.9	27.5
Ltl Niangua R.	41.5	41.5	29.5	32.0	22.5	18.5	5.2	4.9	3.08	3.02	0.07	0.07	15.3	17.3
Mean	44.2	46.1	29.8	32.6	23.8	20.3	4.0	4.2	2.96	2.77	0.09	0.13	21.0	28.0
SD	4.6	3.8	1.8	1.7	3.1	2.5	0.7	0.5	0.10	0.13	0.02	0.03	3.9	7.2
Paired t-test		0.531		0.098		0.016		0.287		0.050		0.132		0.111

Table 6. Differences in metrics between spring and fall 1994, Ozark, cs flow, without chironomids

Streams	Taxa		Family		EPT		Biotic Index		Shannon		Simpson		Dominant	
	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall
Huzzah Cr.	33.5	27.5	22.0	21.0	21.0	15.0	2.7	4.4	2.53	2.27	0.16	0.18	36.0	35.2
Big Cr.	25.0	24.5	20.0	16.5	14.5	11.5	3.7	4.6	2.61	1.97	0.12	0.27	27.0	48.3
Bull Cr.	25.0	24.0	16.5	18.0	12.5	14.5	2.3	4.1	2.25	2.24	0.21	0.26	40.1	42.7
Big Sugar Cr	32.0	21.0	22.0	14.5	20.5	12.5	3.0	3.6	2.64	2.16	0.15	0.22	30.5	41.3
Ltl Niangua	20.5	20.5	15.5	16.0	17.0	12.0	3.4	4.6	2.66	2.59	0.08	0.10	22.3	17.6
Mean	27.2	23.5	19.2	17.2	17.1	13.1	3.0	4.2	2.54	2.25	0.14	0.20	31.2	37.0
SD	4.8	2.5	2.7	2.2	3.3	1.4	0.5	0.4	0.15	0.20	0.04	0.06	6.3	10.6
Paired t-test		0.156		0.283		0.079		0.006		0.075		0.068		0.275

Table 7. Differences in metrics of reference streams in different years (fall 1993 and fall 1994), multi-habitat, Ozark.

Streams	Taxa (1993)	Taxa (1994)	Family (93)	Family (94)	EPT (93)	EPT (94)	Biotic Ind. (93)	Biotic Ind. (94)	Shannon (93)	Shannon (94)	Simpson (93)	Simpson (94)	Dominant (93)	Dominant (94)
Ltl. Niangua	45.0	55.5	29.0	33.0	17.0	18.5	5.6	5.9	3.25	3.32	0.05	0.06	10.5	15.0
Big Sugar	52.0	60.0	31.0	33.0	22.0	22.5	4.6	4.3	3.07	2.97	0.09	0.11	22.0	25.8
Bull	49.0	53.5	25.0	32.0	14.0	18.5	4.5	4.2	2.90	2.88	0.10	0.15	22.1	35.3
Big	56.0	64.0	26.0	33.0	16.0	18.0	4.6	5.7	3.34	3.05	0.05	0.11	11.0	27.7
Huzzah	57.0	74.5	29.0	37.0	18.0	24.0	5.4	5.2	3.26	3.19	0.07	0.08	21.7	19.5
MEANS	51.8	61.5	28.0	33.6	17.4	20.3	5.0	5.1	3.16	3.08	0.07	0.10	17.5	24.7
SD	5.0	8.3	2.4	1.9	3.0	2.8	0.5	0.8	0.18	0.18	0.02	0.03	6.13	7.81
Paired t-test		0.011		0.008		0.046		0.734		0.244		0.060		0.103

Table 8. Differences in metrics of reference streams in different years (fall 1993 and fall 1994), cs flow, Ozark.

Streams	Taxa (1993)	Taxa (1994)	Family (93)	Family (94)	EPT (93)	EPT (94)	Biotic Ind. (93)	Biotic Ind. (94)	Shannon (93)	Shannon (94)	Simpson (93)	Simpson (94)	Dominant (93)	Dominant (94)
Ltl Niangua	18	23.5	13	17.0	9	12.0	4.4	4.5	2.29	2.72	0.14	0.09	29.1	16.6
Big Sugar	20	24.0	15	15.5	12	12.5	3.1	3.3	2.14	2.25	0.23	0.20	45.3	40.1
Bull Cr.	25	28.5	13	19.0	11	14.5	3.5	3.6	2.41	2.40	0.17	0.19	34.3	40.5
Big Cr.	30	24.5	20	17.5	13	11.5	4.0	5.0	2.81	2.08	0.09	0.25	16.8	46.8
Huzzah Cr.	30	41.0	16	22.0	12	15.0	5.2	4.9	2.86	2.72	0.07	0.13	11.3	29.2
Mean	24.6	28.3	15.4	18.2	11.4	13.1	4.0	4.3	2.50	2.43	0.14	0.17	27.4	34.6
SD	5.5	7.4	2.9	2.5	1.5	1.6	0.8	0.8	0.32	0.29	0.06	0.07	13.6	11.9
Paired t-test		0.236		0.167		0.150		0.369		0.736		0.435		0.398

multihabitat datasets (Fig. 1), Total taxa and EPT showed high separation among time periods, while Biotic and Shannon's indices are more similar. For the single habitat datasets (Fig. 2) results are similar. The within-year samples of 1993 are most similar, while the 1995 summer metrics are most different. If 1995 data were to be used as a standard, REF sites from other years would likely be classed as degraded (e.g., fall 1993 Total taxa). Either year-to-year natural variability is great or summer has a different fauna than spring and fall.

CONCLUSION

Until further temporal data is collected and evaluated, we recommend that REF sites be sampled each year that degraded sites are sampled. Although this would require additional resources and effort, our results have shown that a small subset of REF sites (perhaps 5-10) is all that is necessary to establish baseline conditions. The alternative is to "average out" metrics from REF sites over a period of seasons and years and use those scores to develop the SCI. This approach will, however, result in a decrease in sensitivity and in the ability to detect degraded conditions.

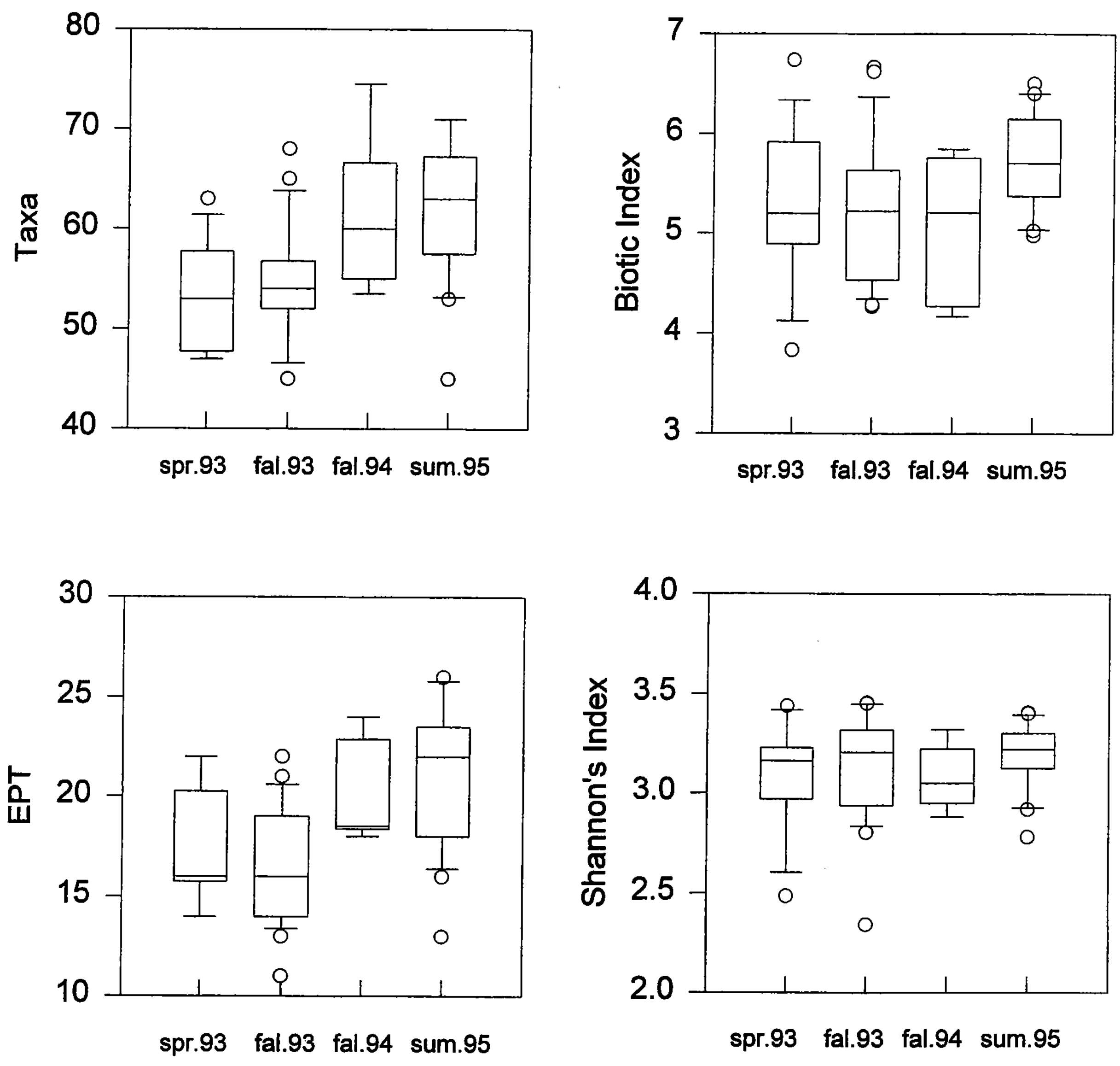


Fig. 1. Box plots for the four core metrics illustrating temporal differences: Ozark ecoregion and multihabitat data.

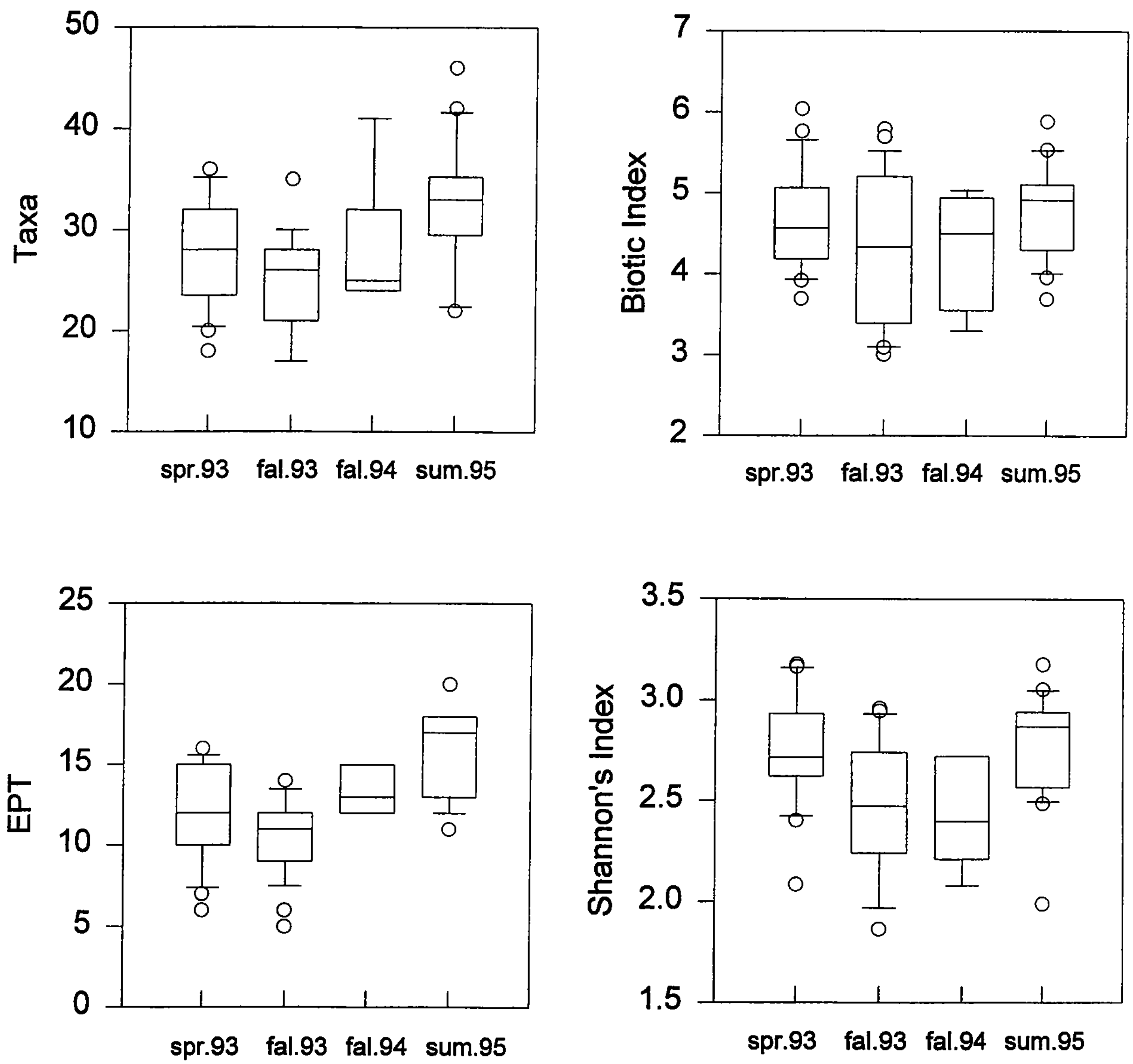


Fig. 2. Box plots for the four core metrics illustrating temporal differences: Ozark ecoregion and cs flow.

Chapter 13

ARE CHIRONOMIDAE NECESSARY?

INTRODUCTION

This family of nonbiting midges is ubiquitous in all aquatic systems, and often comprises numerically the most dominant group. Some genera are known to be particularly tolerant to pollution and have long been indicators of problems, e.g., bloodworms of the subfamily Chironominae. Identification of Chironomidae below the Family level is quite laborious because each animal has to be mounted on a microscope slide, cleared with chemicals, and examined under high magnification. Identification of Chironomidae can occupy up to half of the total time spent identifying the entire sample. If it were not necessary to identify Chironomidae to genus, theoretically twice as many sites could be evaluated.

OZARK ECOREGION

We reanalyzed our summer 1995 dataset, where REF and degraded streams were paired and comparisons were made of REF to HAB and REF to ORG. We evaluated the sensitivity of several metrics both with and without Chironomidae being identified to the genus taxonomic level.

Results

Mean Metric Differences

Multihabitat (cs flow + nonflow). When comparisons were made of with to without chironomidae for REF-HAB, the dataset without Chironomidae performed equal to or better than the dataset with Chironomidae for every metric tested (Table 1). The REF-ORG comparison indicated the without Chironomidae dataset performed equally or better for all metrics except the BI, where the difference was minor.

Single Habitat. Similar results were obtained using single habitat data (cs flow). The without-Chironomidae dataset performed equally to, or better than, the with-Chironomidae dataset in all instances except one.

Paired Metrics

A comparison of results with and without Chironomidae was done on the summed dataset for the three paired metrics (Table 2). Using the impairment threshold based on replicated REF sites of 1993, we see, in the vast majority of cases, close correspondence between values obtained with or without Chironomidae. For multihabitat data, only one of the QSI, two of the PPSI, and one of the CCL comparisons would give a different interpretation of impairment. For cs flow data, none of the QSI, none of the PPSI, and only two of the CCL comparisons would give different interpretations of impairment.

Box Plots

Box plots were constructed for the 1995 summer multihabitat data with and without Chironomidae. For the REF-HAB comparisons (Fig. 1) all metrics showed the same sensitivity with and without Chironomidae, except Total taxa where the without Chironomidae data were more discriminating, and Shannon's diversity index, where the with Chironomidae data was a better discriminator. For REF–ORG comparisons (Fig. 2) the same sensitivities

Table 1. A comparison of the sensitivity of metrics with (All taxa) and without (w/o) Chironomidae. Values are p-values from paired t-tests comparing reference to degraded streams. Data are for paired streams, Ozark Ecoregion, summer 1995.

	Taxa	Family	EPT	BI	Shannon	Simpson	% Dom
Multihabitat							
REF-HAB							
All taxa	0.186	0.025	0.678	0.424	0.096	0.100	0.206
w/o Chironomidae	0.103	0.025	0.678	0.111	0.049	0.044	0.042
REF-ORG							
All taxa	0.035	0.027	0.003	0.009	0.018	0.027	0.112
w/o Chironomidae	0.007	0.027	0.003	0.011	0.006	0.019	0.059
Single Habitat (cs flow)							
REF-HAB							
All taxa	0.390	0.128	0.607	0.224	0.034	0.006	0.005
w/o Chironomidae	0.208	0.128	0.607	0.291	0.024	0.006	0.002
REF-ORG							
All taxa	0.015	0.004	0.000	0.006	0.035	0.133	0.262
w/o Chironomidae	0.001	0.004	0.000	0.011	0.010	0.029	0.029

Table 2. Paired metrics, Ozark, summer 1995 (data with [w] and without [w/o] chironomids).

			Multihabitat						cs flow					
			QSI		PPSI		CCL		QSI		PPSI		CCL	
Ref. sites	Hab.		w	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w	w/o
Ltl. Niangua	Dry Aug.(C)	1	37	43	0.41	0.44	0.53	0.54	41	43	0.22	0.29	0.46	0.53
Starks	Greasy	2	44	39	0.41	0.43	0.46	0.50	55	58	0.49	0.49	0.47	0.54
Deer	Cole Camp	4	37	35	0.37	0.31	0.25	0.39	44	44	0.30	0.24	0.22	0.27
Woods Fk.	Clark	5	53	69	0.48	0.55	0.31	0.47	49	54	0.44	0.47	0.33	0.47
E Fk. Huzz.	Hutchins	6	48	50	0.44	0.46	0.39	0.33	39	39	0.44	0.43	0.44	0.39
Grand Pond	Crooked	7	33	33	0.37	0.32	0.51	0.39	37	37	0.36	0.35	0.29	0.27
Huzzah	Big cr.. (Iron)	8	49	50	0.43	0.43	0.37	0.46	51	51	0.43	0.41	0.44	0.44
Meramec	Indian	9	64	66	0.47	0.47	0.30	0.33	48	48	0.47	0.46	0.41	0.38
Maries	Ltl. Tavern	10	39	39	0.24	0.26	0.44	0.57	35	36	0.33	0.39	0.73	0.81
Ref.	Org.													
Swan	Clear	1	10	3	0.28	0.35	1.58	2.58	12	1	0.22	0.08	2.40	3.50
Big Sugar	Turkey	2	19	14	0.33	0.36	1.35	1.06	9	2	0.04	0.03	2.70	4.60
Lindley	Piper	3	37	42	0.44	0.48	0.21	0.33	19	22	0.46	0.44	0.31	0.69
W. Piney	Hominy	4	32	35	0.41	0.41	0.83	0.89	42	40	0.49	0.49	1.21	1.12
Whetstone	E. Fk. Whets	5	8	6	0.26	0.06	1.00	1.65	5	3	0.61	0.05	1.43	3.00
Shawnee	Ltl. Lindley	6	25	26	0.33	0.54	1.38	2.64	11	8	0.27	0.24	1.45	2.60
N. Jacks	Dry Aug. (L)	7	39	45	0.41	0.40	0.56	0.58	15	23	0.32	0.33	0.59	0.77
Marble	Spring	8	28	27	0.37	0.35	0.66	0.81	40	41	0.36	0.36	0.82	0.80
Impairment threshold based on replicate reference sites, 1993				60		0.5		0.5		60		0.5		0.5

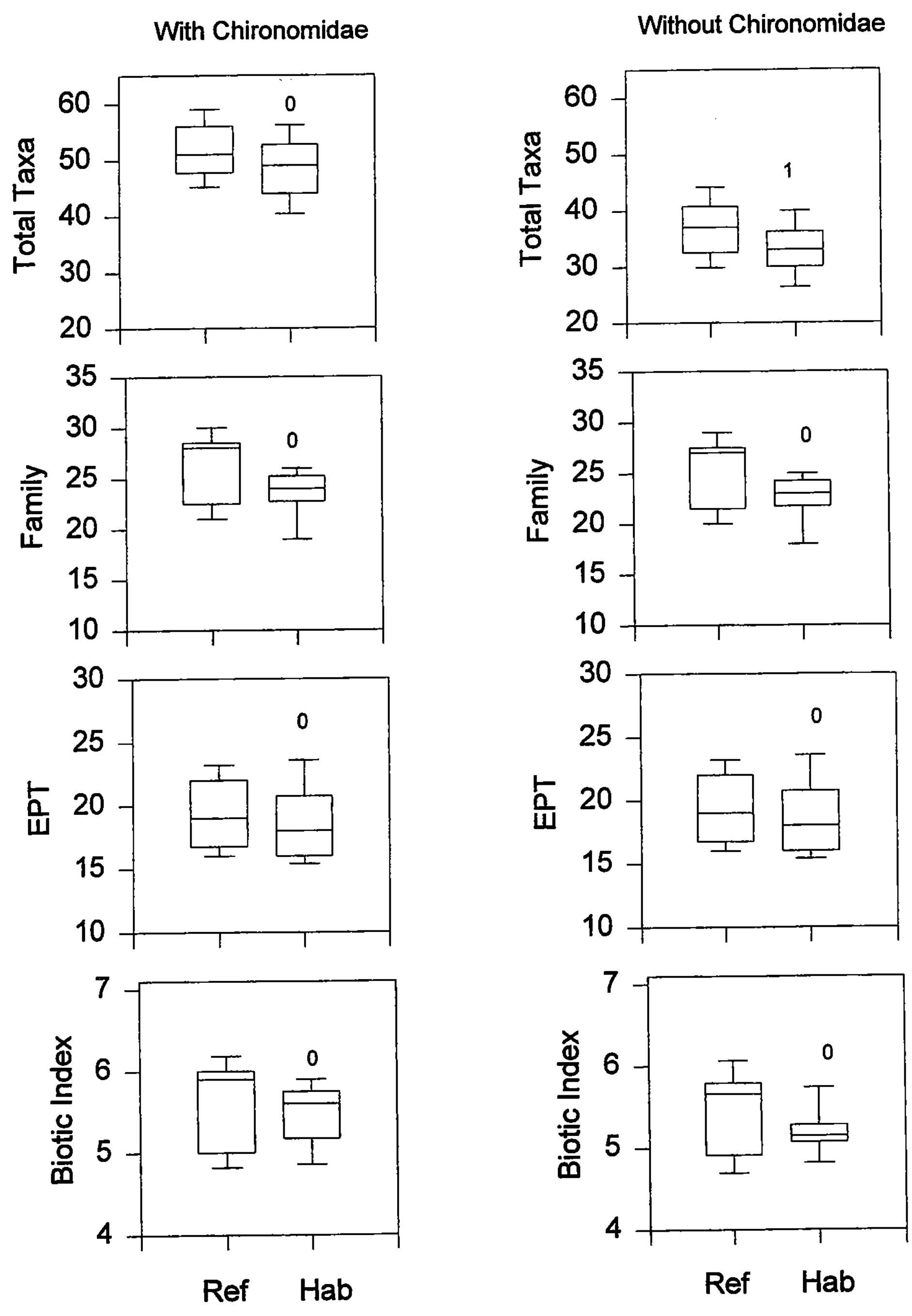


Fig. 1. Box plot comparisons for metrics calculated with Chironomidae (left column) and without Chironomidae (right column). Data for multihabitat, from summer 1995.

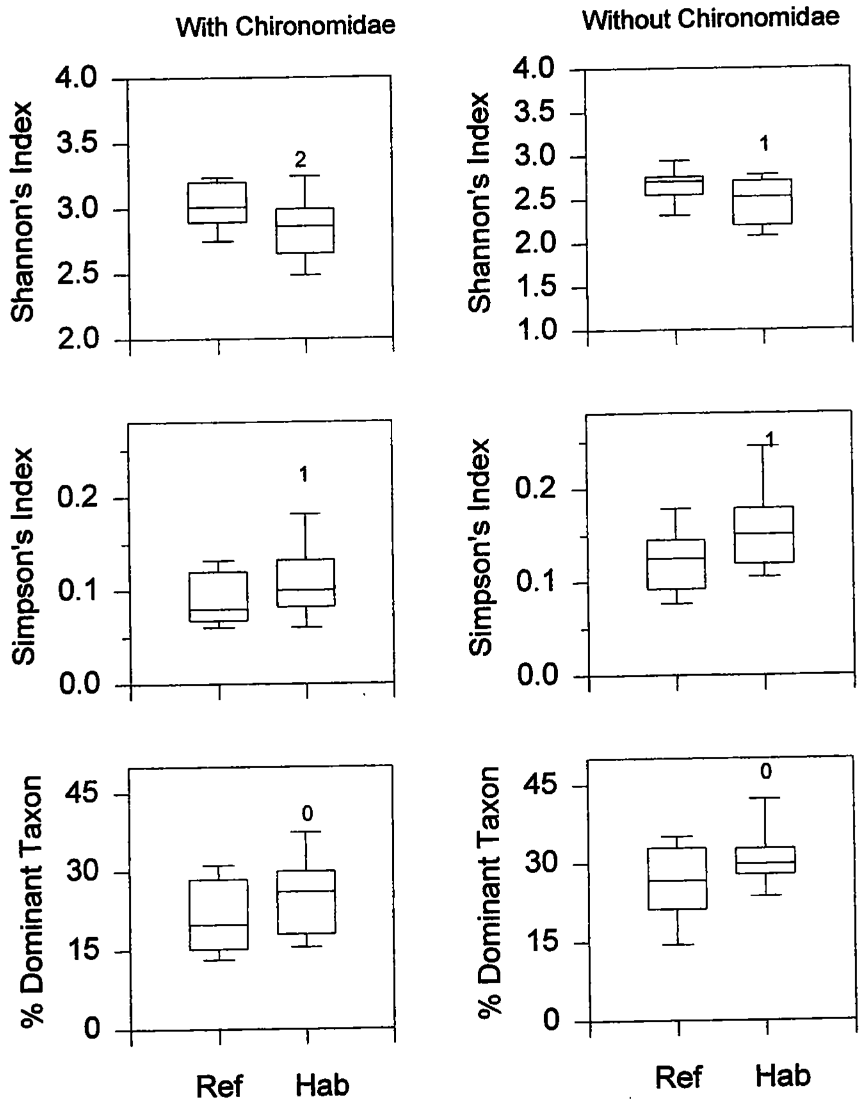


Fig. 1. Continued.

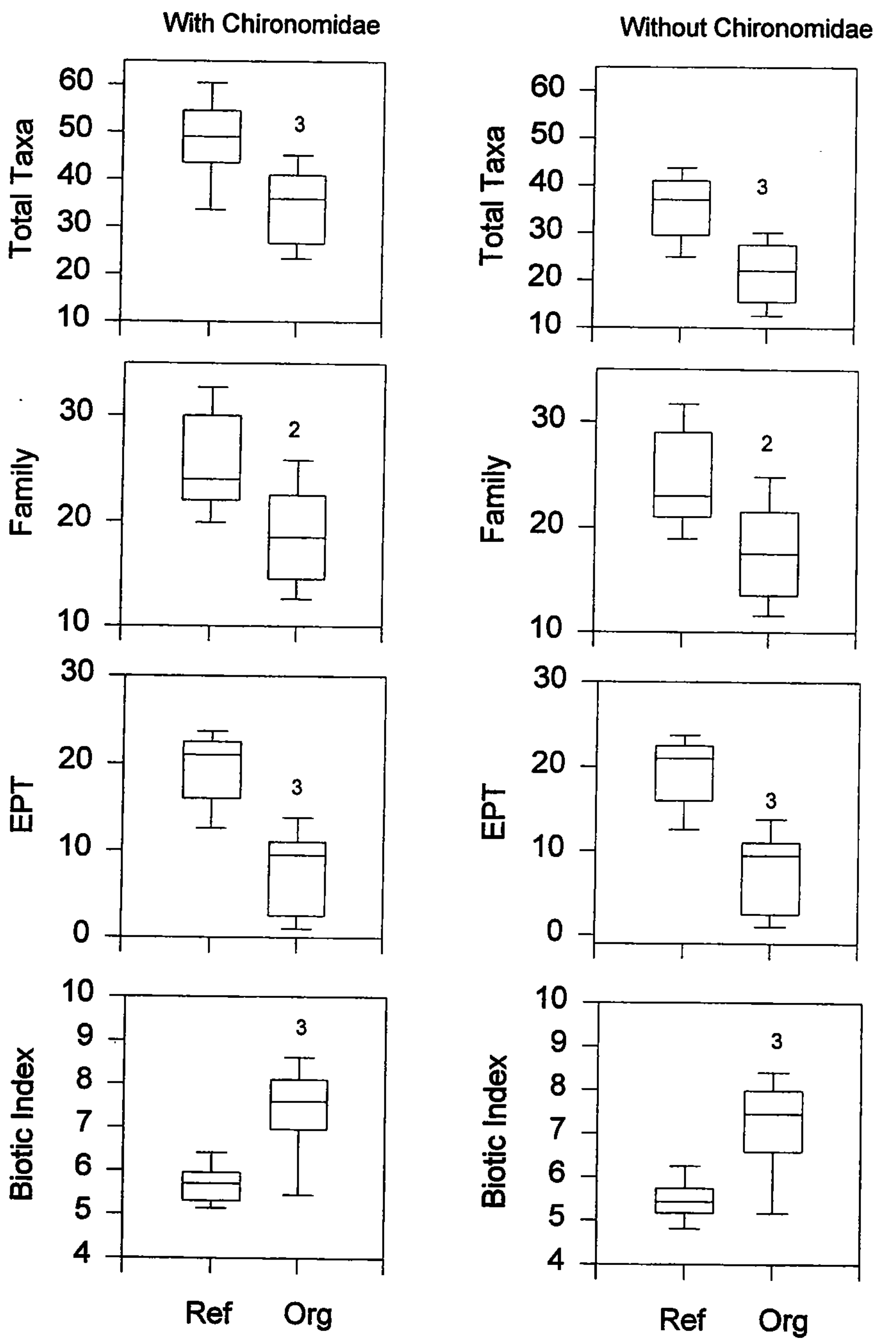


Fig. 2. Box plot comparisons for metrics calculated with Chironomidae (left column) and without Chironomidae (right column). Data for multihabitat from summer 1995.

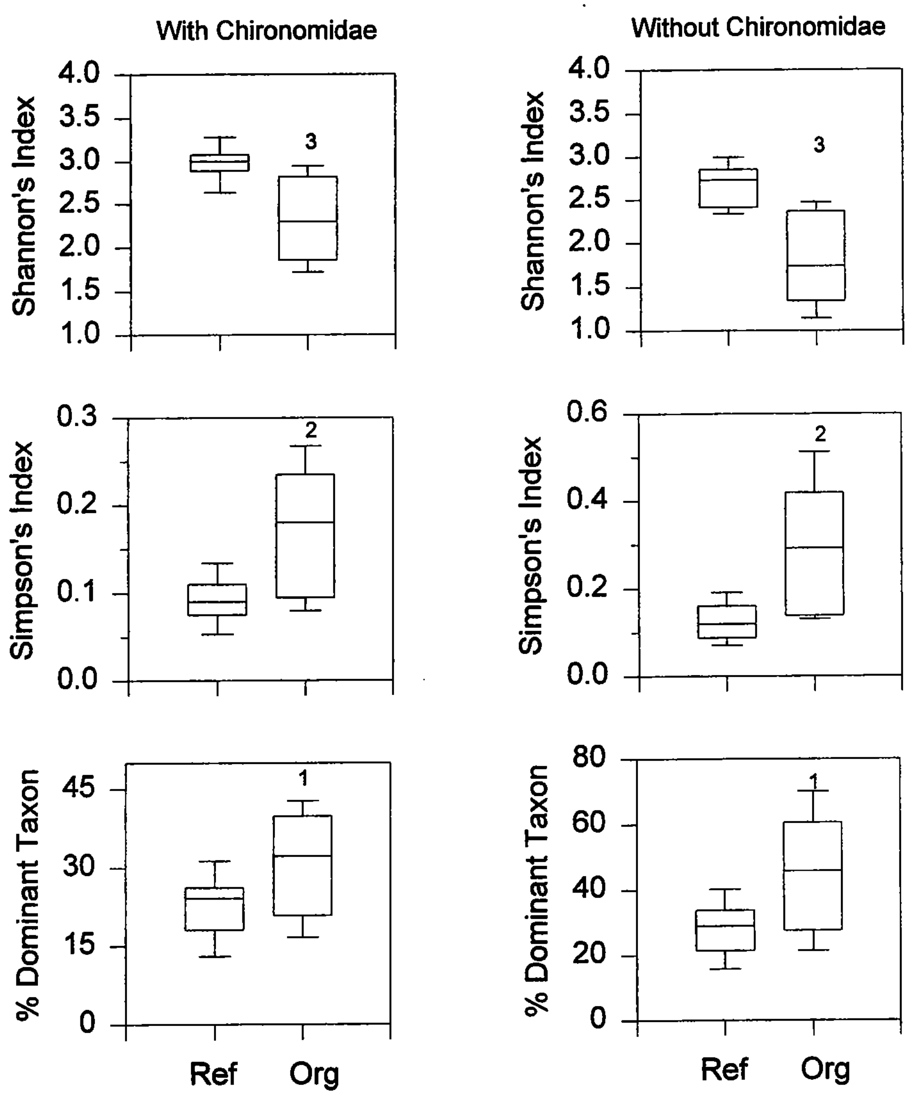


Fig. 2. Continued.

were found for each metric whether it was with or without Chironomidae.

Box plots were constructed for the same 1995 data using just a single habitat (cs flow) with and without Chironomidae. For the REF-HAB comparisons (Fig. 3) all metrics except one showed identical sensitivities with and without Chironomidae. The only exception was the Biotic Index, which showed slight sensitivity when Chironomidae were included but no sensitivity when the midges were excluded.

For the REF-ORG comparisons, identical sensitivities were obtained for each metric regardless whether or not chironomidae were included (Fig. 4).

PRAIRIE ECOREGION

We reanalyzed the fall 1994 Prairie Ecoregion data set. We evaluated the sensitivity of several metrics with and without Chironomidae.

Results

Mean Metric Differences

Multihabitat. When statistical comparisons were made for both REF-HAB and REF-ORG data sets the data without Chironomidae performed equal to or better than the data set with Chironomidae for every metric tested (Table 3).

Single Habitat (non flow). Results similar to the multihabitat data set were obtained using the single habitat data. That is, metrics calculated without Chironomidae had equal or better sensitivity than with the Chironomidae metrics (Table 3).

Box Plots

Multihabitat. Box plots were constructed for the fall 1994 multihabitat data sets with and without Chironomidae (Fig. 5). For REF-HAB comparisons all 5 metrics that could show a difference (EPT cannot change and Family is unlikely to change) were more sensitive using the without Chironomidae data set. For REF–ORG comparisons, 3 of the 5 metrics that could show a difference were more sensitive using the without Chironomidae data set.

Single Habitat (non flow). For the Prairie Ecoregion using only single-habitat data, both REF-HAB and REF-ORG results were consistent with previous analyses where the without Chironomidae data set performed better than the with Chironomidae data set (Fig. 6).

CONCLUSION

We conclude that without–chironomid data showed similar or better results than with-chironomid data. Comparing the results from multi- and single habitat data without chironomids, there were few differences, although in some cases, single habitat sampling showed better results than multihabitat sampling. Therefore, for both the Ozark and Prairie regions, a single habitat sampling analyzed without the Chironomidae is sufficient.

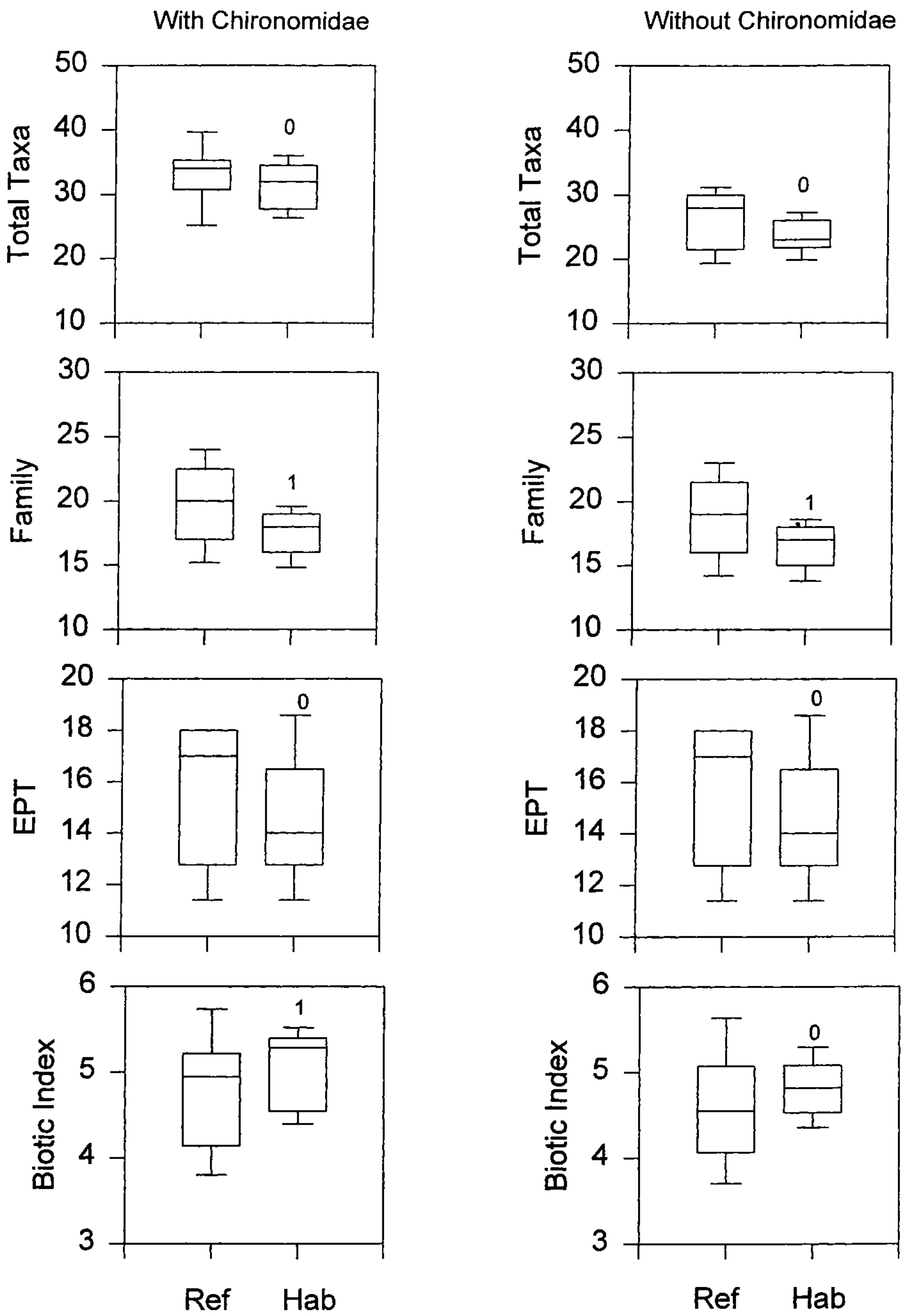


Fig. 3. Box plot evaluations of habitat-altered sites, comparing metrics calculated with Chironomidae (left column) and without Chironomidae (right column). Data for single habitat from summer 1995, Ozark region.

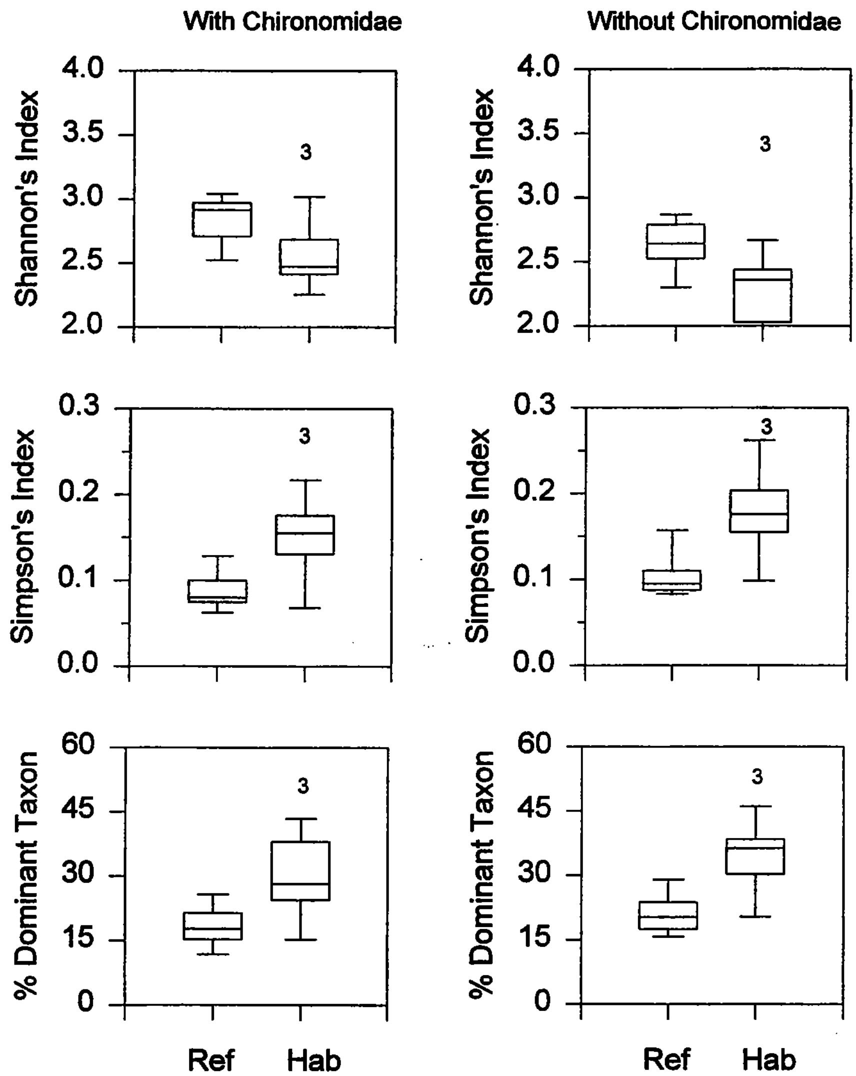


Fig. 3. Continued

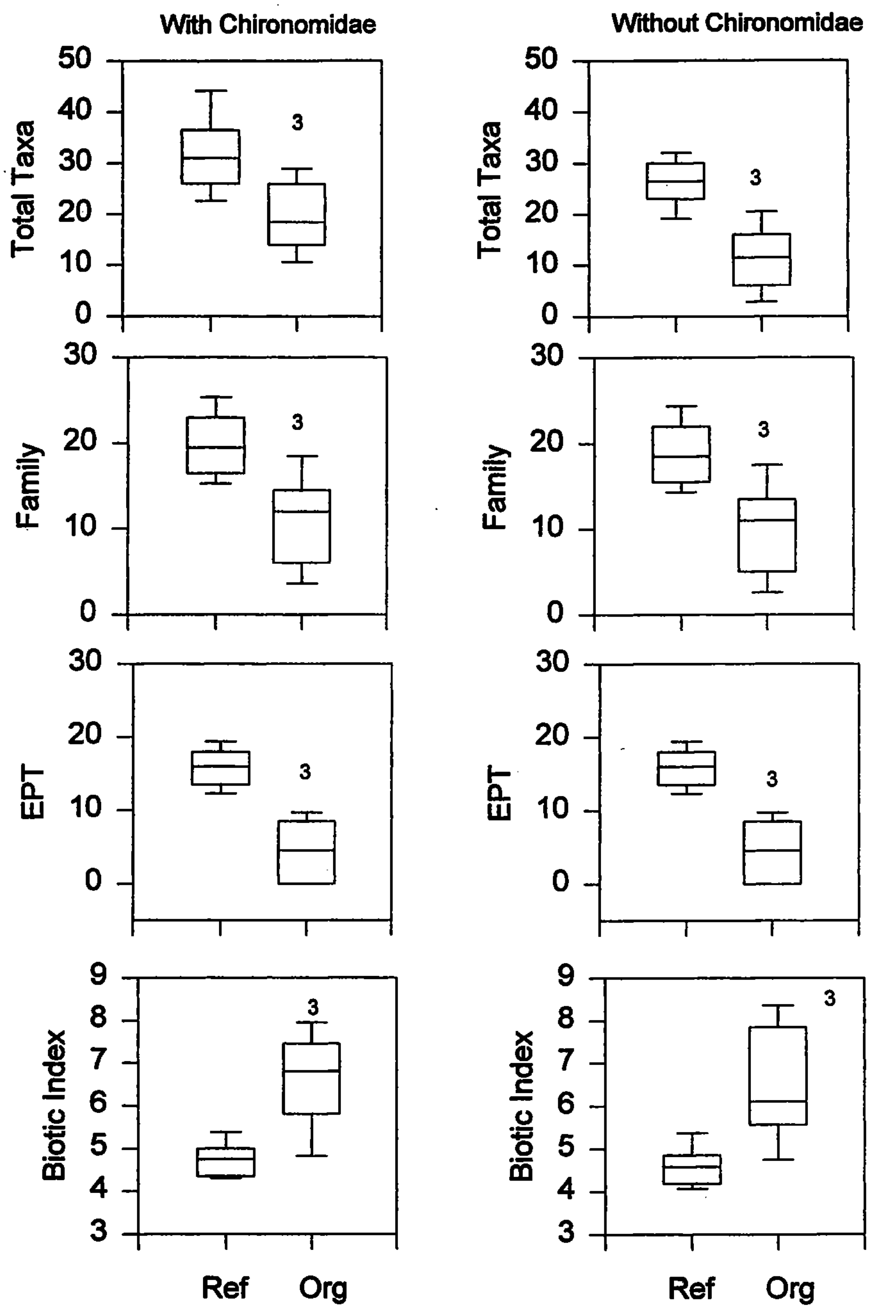


Fig. 4. Box plot evaluations of organically enriched sites, comparing metrics calculated with Chironomidae (left column) and without Chironomidae (right column). Data for single habitat from summer 1995, Ozark region.

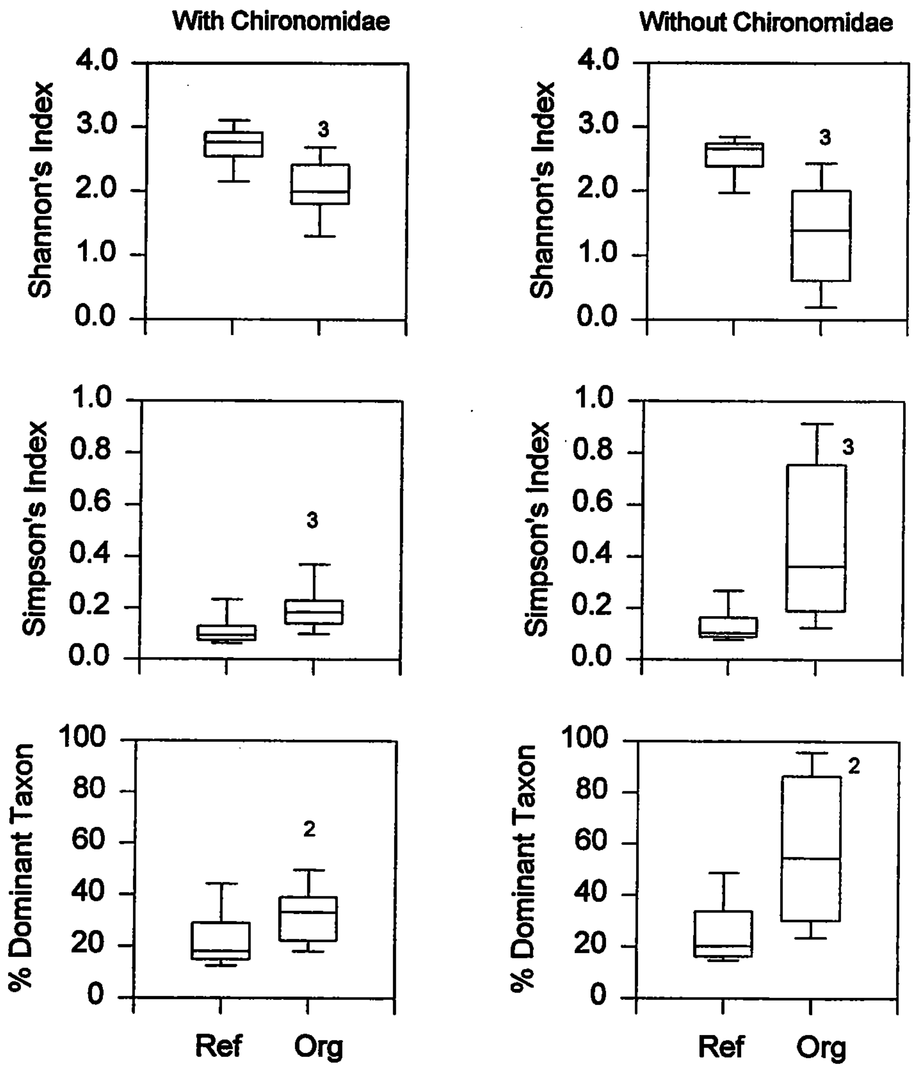


Fig. 4. Continued

Table 3. A comparison of the sensitivity of metrics with (All taxa) and without (w/o) Chironomidae. Values are p-values from paired t-tests comparing reference to degraded streams. Data are for Prairie Ecoregion, fall 1994.

	Taxa	Family	EPT	BI	Shannon	Simpson	% Dom
Multihabitat							
REF-HAB							
All taxa	0.290	0.032	0.046	0.088	0.142	0.301	0.801
w/o Chironomidae	0.008	0.051	0.046	0.012	0.050	0.120	0.330
REF-OR							
All taxa	0.897	0.429	0.159	0.114	0.244	0.360	0.310
w/o Chironomidae	0.216	0.404	0.159	0.009	0.154	0.331	0.560
Single Habitat (non flow)							
REF-HAB							
All taxa	0.839	0.246	0.201	0.105	0.587	0.923	0.450
w/o Chironomidae	0.414	0.167	0.201	0.040	0.098	0.456	0.879
REF-OR							
All taxa	0.581	0.250	0.007	0.030	0.219	0.286	0.378
w/o Chironomidae	0.067	0.174	0.007	0.005	0.128	0.291	0.510

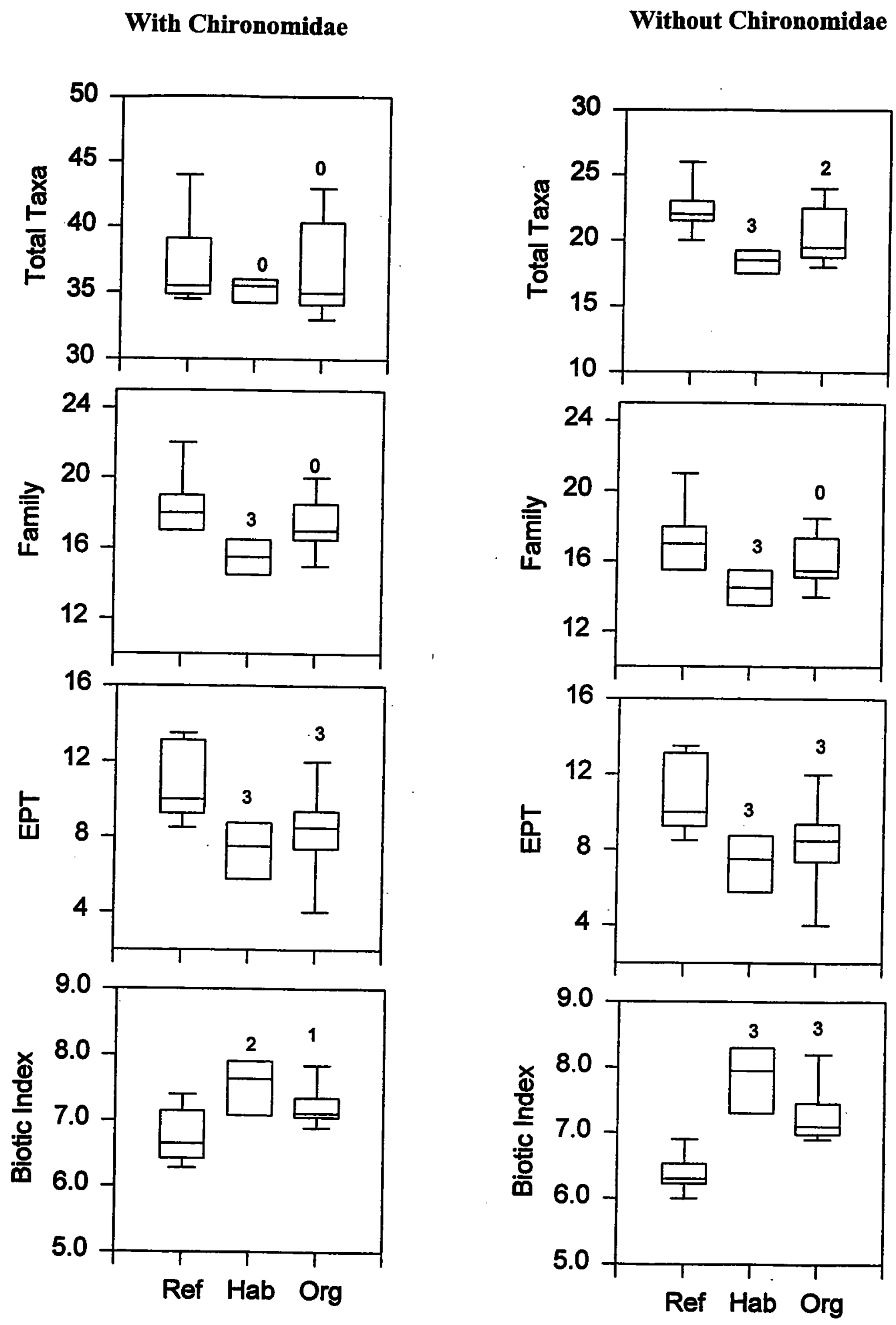


Fig. 5. Box plot evaluations of organically enriched sites (ORG) and habitat-altered sites (HAB) to reference sites (REF), comparing metrics calculated with Chironomidae (left column) and without Chironomidae (right column). Data for multihabitat, 1994, Prairie region.

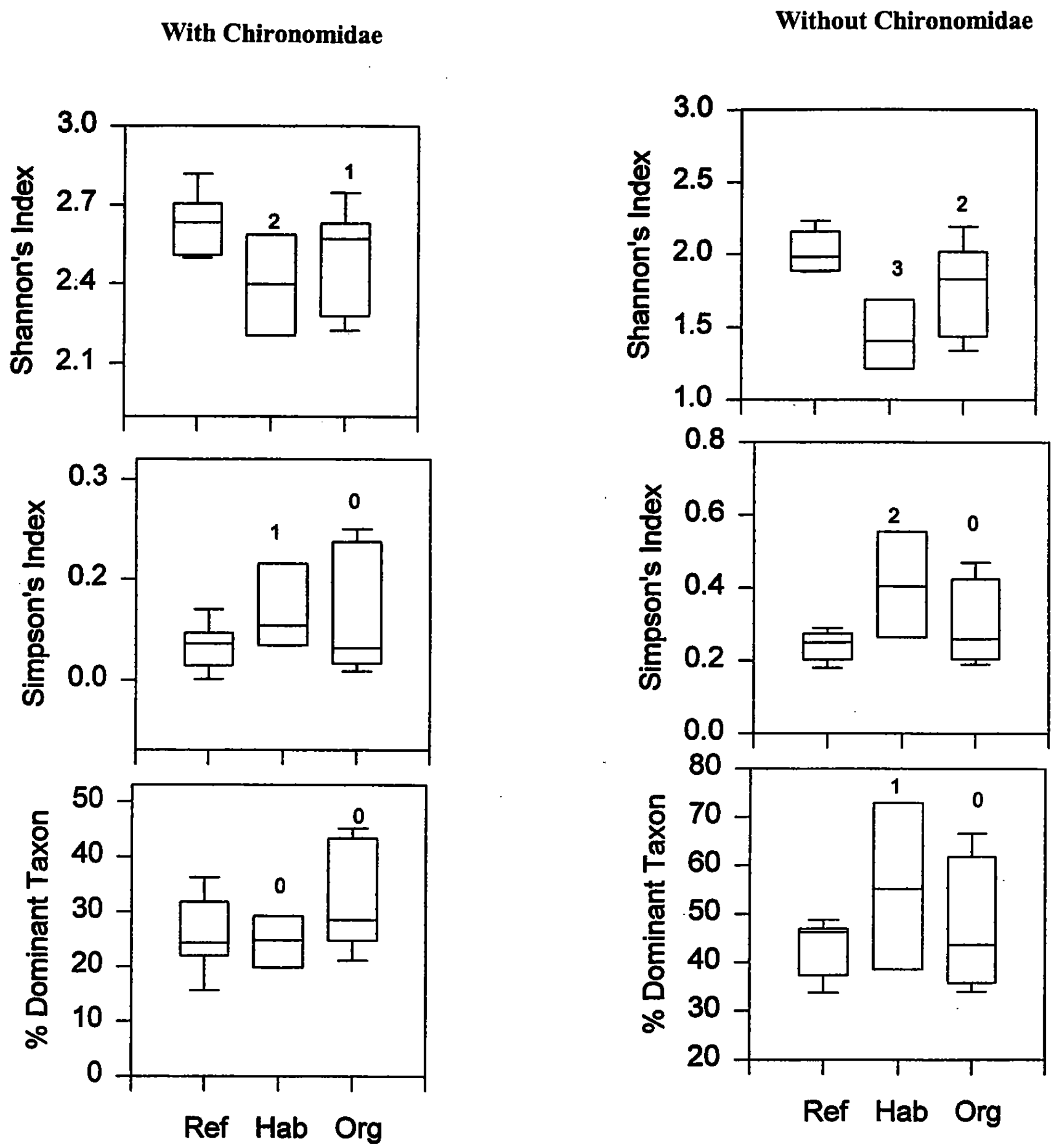


Fig. 5. Continued

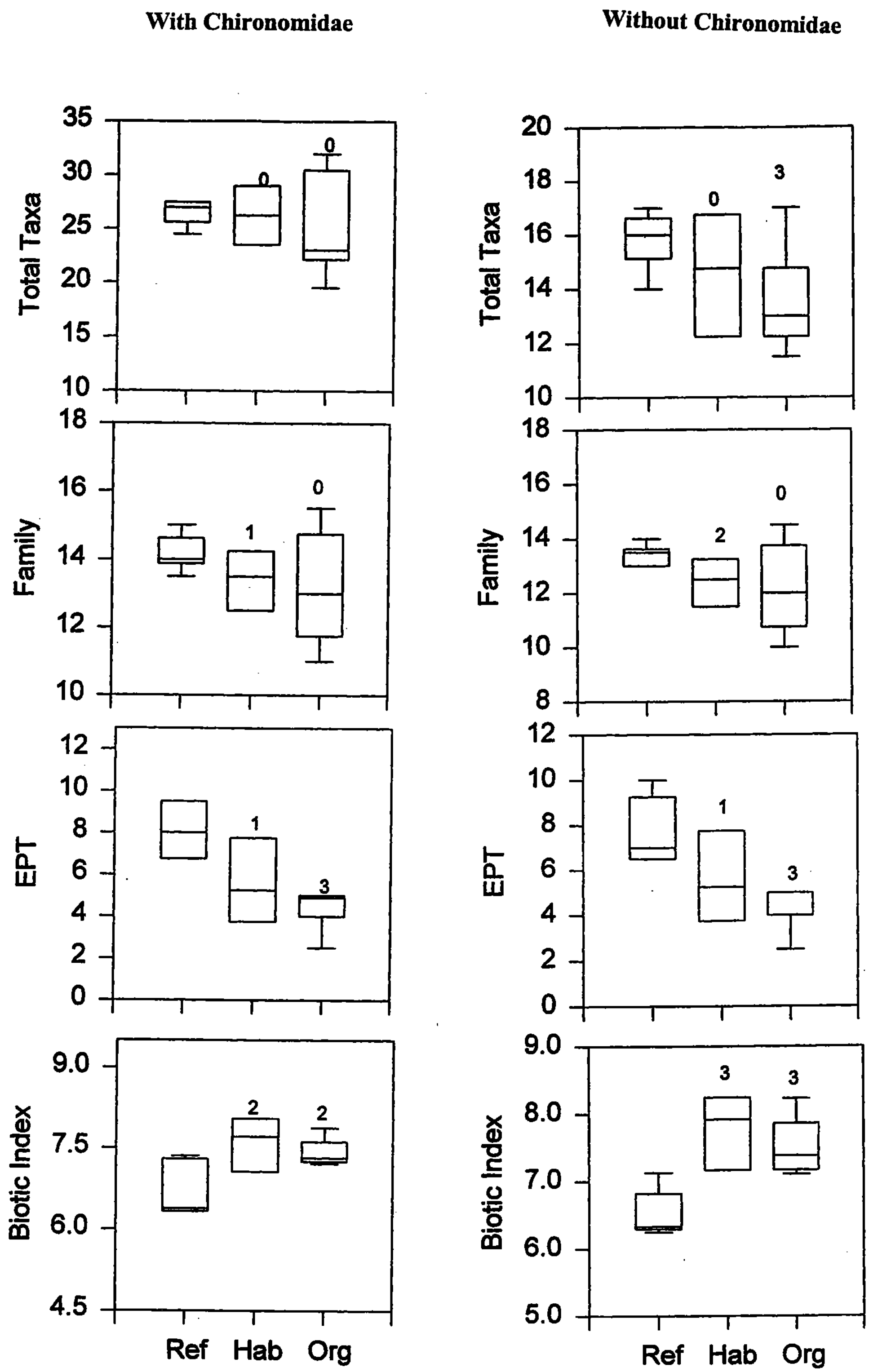


Fig. 6. Box plot evaluations of organically enriched sites (ORG) and habitat-altered sites (HAB) to reference sites (REF), comparing metrics calculated with Chironomidae (left column) and without Chironomidae (right column). Data for single habitat - non flow, 1994, Prairie region.

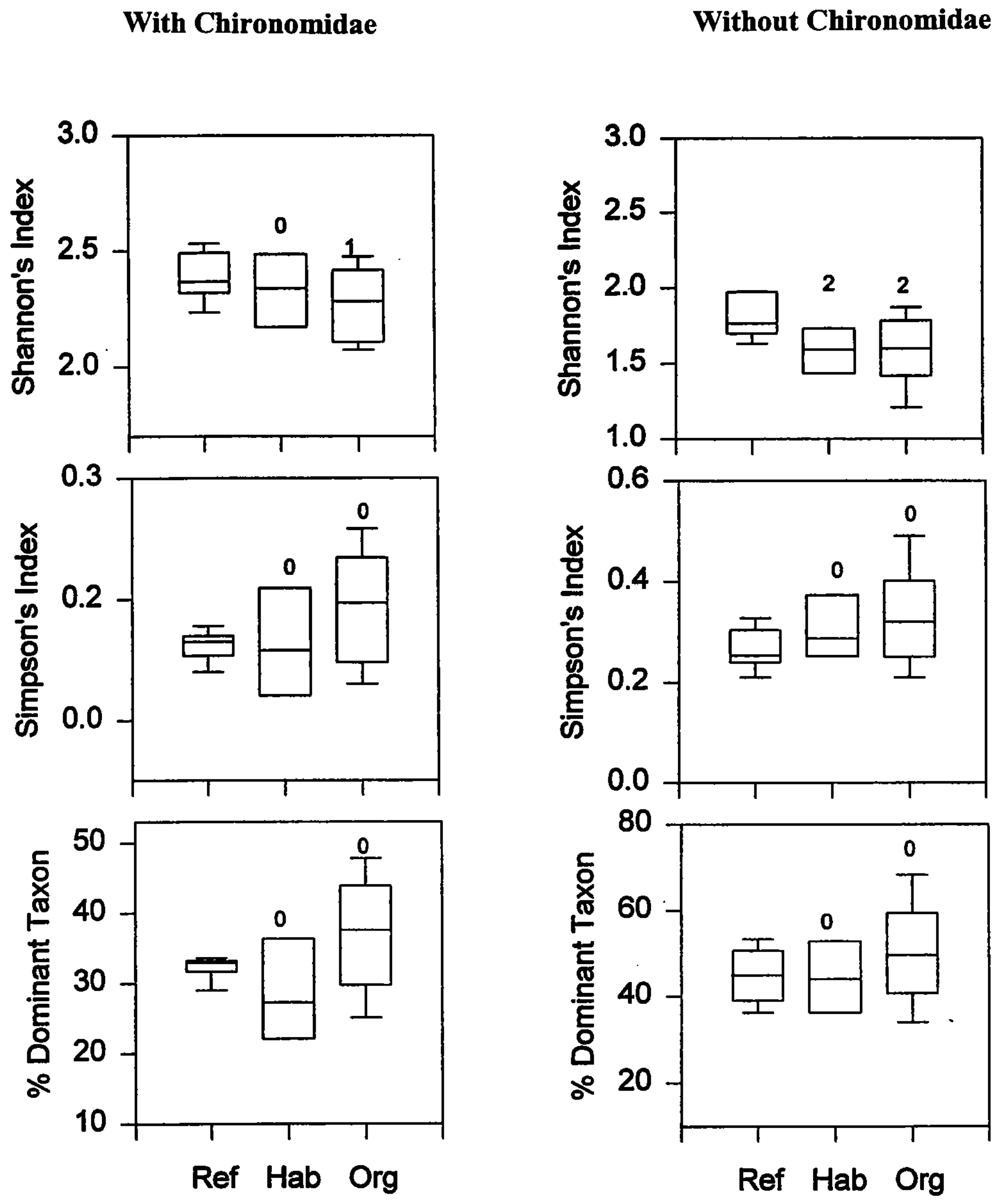


Fig. 6. Continued

Chapter 14

FISH AS BIOMONITORS

INTRODUCTION

Stream quality monitoring programs are committed to detection of impacts of water quality or habitat alteration on lotic communities. The ability to detect authentic impacts (i.e., those not the result of sampling biases) depends upon quality of the data to be analyzed (Toft and Shea 1983). Unfortunately, natural resource professionals are rarely afforded the opportunity to examine the adequacy of their data and revise their sampling protocol, if necessary. This can lead to the inability to detect significant impacts on stream communities until it is large and potentially irreversible. Gear bias and sample variance are two main factors that influence the ability to detect impacts. Gear bias can influence the ability to detect phenomena by obfuscating significant impacts on stream quality (Bayley and Dowling 1993), while high sample variance influences the ability to statistically detect phenomena (Gold 1969). Therefore, it is essential to evaluate sources of gear bias and sampling variance in order to develop a sampling strategy that can meet predetermined study objectives.

Most fish collection methods are selective to some extent. The catchability of fish has been attributed to differences in size, body shape or morphology, species specific behaviors, or a combination of behavioral and morphological traits (Bagenal 1979, Reynolds 1983, Lyons 1986, Bayley and Dowling 1990). In addition, physical and chemical characteristics of a stream reach such as water conductance, turbidity, width, depth, velocity, and physical structures (e.g., vegetation, snags, boulders) individually and in combination, can also affect catchability (Rodgers et al. 1992, Bayley and Dowling 1993). The quality or health of a stream can be characterized by the structure of the fish community (Karr 1981), which is influenced by physical (e.g., habitat) and chemical (e.g., water quality) stream attributes. Therefore, the physicochemical variables that influence the quality of a stream can also be the same factors that affect capture efficiencies. For instance, if only a few fish were collected in a reach with deep water (e.g., deeper than the electrofishing field), was this a reflection of stream quality or of gear efficiency? Failure to account for differences in efficiency, when making comparisons among sites with different physicochemical characteristics may introduce a systematic error or bias into the data. Thus, sampling bias could have serious consequences on the interpretation of fish data used to assess stream quality.

Estimates of sampling biases can be obtained by conducting gear efficiency evaluation procedures and modeling the collection efficiency of each method (Bayley and Dowling 1990, Rodgers et al. 1992, Riley et al. 1993). Unbiased estimates of fish abundance can then be obtained by adjusting raw catch data with gear efficiency models. However, calibrating gear efficiency is an expensive, time consuming process. Therefore, it would be more economical to utilize a collection gear for which sampling biases are known, applied under the circumstances in which catchability is reliable.

As discussed above, high variance is one factor that influences the ability to statistically detect phenomena (Gold 1969) and must also be considered when developing a sampling protocol for monitoring or evaluating stream quality. Variance is influenced by factors such as number of samples collected and how the samples are apportioned in time and space. Peterson and Rabeni (1995) suggest that optimal sampling strategies include collection of samples from several locations within a stream during a single late-summer time period, but caution that study specific differences such as gear choice may alter sample size requirements. Therefore, it is essential to determine the number of samples required to meet predetermined study objectives.

METHODS

The DC backpack electrofisher and minnow seine are two fish collection gear for which efficiencies have been thoroughly evaluated on streams (Bayley et al. 1989, Bayley and Dowling 1990). In addition to having efficiency models, both gear are relatively easy and inexpensive to operate which is also a desirable characteristic for sampling gear. Backpack electrofishers are portable, require only two persons to operate, and consist of a power source (e.g., battery), transformer, hand held anode, and trailing cathode. Minnow seines are also very portable and require two persons to operate. Consequently, we chose to evaluate effectiveness of these two gear for detecting impacts of water quality or habitat alteration on stream fish communities.

Fish Sampling

To evaluate sampling strategies for fish communities, two-five stream reaches were blocked off with 6 mm mesh nets. A reach was defined as a stream segment containing a pool, run, and riffle sequence (Frissell et al. 1986). Pool-riffle sequences are repeatable hydrologic features with a periodicity of approximately five to seven times the mean stream width (Gordon et al. 1992). Therefore, in streams without well defined pool-riffle patterns, a reach was considered to be six times the mean stream width. Fishes were collected from within the blocked off area with either a 30 ft minnow seine with 6 mm mesh or a DC backpack electrofisher operating at 220 V and 5 A. Both gear used a two pass procedure the first upstream, the second down. Both gear were operated in such a manner that sampling simulated a nonblocked off area (i.e., fishes were not herded into or trapped against the blocknets). Fish data from each reach was kept separate to facilitate analysis of sampling variance (below).

To verify the minnow seine and backpack electrofisher efficiency models (Bayley and Dowling 1990), fishes collected with the above procedure were identified, marked with a small fin clip that did not impair swimming ability, and total length measured. Marked fish were allowed to recover for at least 20 min in ambient stream water and released into the blocked off area. Great care was taken in handling fish, and only fish that had recovered sufficiently were released. In addition, the stream and blocknets were checked immediately before sampling to ensure that no fish had been affected by the marking procedure or become trapped in the net. After a dispersal period (>20 min), fishes were collected with a secondary gear (i.e., minnow seine or backpack electrofisher) that was not used to sample the fishes initially. The secondary sample consisted of fishes collected in two passes, the first upstream and the second downstream. All fish collected with the secondary gear were identified to species, and total lengths were measured and rounded down to the nearest millimeter. Large fish (>100 mm) and centrarchids were identified, measured in the field, and released. Small fish were preserved in 10% formalin and taken to the laboratory to facilitate more accurate identification and measurement.

Physical Measurements

Several physical and chemical stream characteristics known to affect the efficiency of the backpack electrofisher and minnow seine (Bayley and Dowling 1990) were measured in each blocked off site before or immediately following fish collection. Water conductance, temperature, and turbidity were measured in the middle of the site. Mean water velocity and depth were determined by averaging readings at 5-10 points within a site. Velocity was measured with a water current meter attached to a standard top-set wading rod and measured at 0.6 depth where depth <2 ft; at greater depths the average of velocities at 0.2 and 0.8 depth were used. Percentage of the site covered with vegetation and percentage of the site containing riffles were visually estimated. Physical impedance was also assessed. Objects that prevented complete sampling of a blocked off site such as large snags, boulders, and overhanging trees determined the value of physical impedance that scored from 0 = none to 3 = heavy.

Minnow seine efficiency models use the derived variable percentage of the area sampled (PAS) which is calculated as PAS = (S/W)*N*100 where S is the seine length, W the mean stream width in feet, and N is the number of passes with the seine (i.e., N = 2 when an up and downstream pass is made). The first term (S/W) is 1 when the mean stream width is less than the seine length.

Definitions and Statistical Analysis

Measured efficiency (E) was determined for each species group as E = R/M where R is the number of recaptured fish and M is the number of marked fish in a blocked off area. Predicted efficiency was calculated by applying the Bayley and Dowling (1990) efficiency models for two runs (Tables 1 and 2) as:

$$\pi = \{1 + \exp(-(\beta 0 + \beta 1 x1 \ldots))\}^{-1} \quad (1)$$

where π = predicted efficiency as a fraction
$\beta 0$ is the constant
$\beta 1$ etc., are the model coefficients
$x1$ etc., are the corresponding variable values.

The corresponding upper 95% confidence limit was calculated as:

$$\pi\ (\text{upper}) = \{1+\exp(-(\ln(\pi/(1-\pi)) + 1.96\sqrt{(\{m\pi(1-\pi)\}^{-1} + \sigma^2)}))\}^{-1} \quad (2)$$

where π = estimated efficiency, from (1) above
m = number of marked fish
σ^2 = extra-binomial variance.

The lower confidence limit was obtained by changing the sign preceding 1.96.

Efficiency estimates were not available for sculpin (Cottidae; Bayley and Dowling 1990). However, catfish (Ictaluridae) efficiency estimates were available. Sculpins and catfish are bottom dwelling fishes that occupy a variety of similar habitats and have fairly similar body shapes (Pflieger 1975). Consequently, we used the catfish efficiency model to predict the efficiency for sculpin.

Effectiveness of the efficiency models was evaluated by inspecting plots of measured and predicted efficiency, with 95% confidence limits, for species groups (Table 3). Raw catch data for the remainder of the analysis were adjusted by dividing length-frequency fish data with the corresponding π from the above equation.

Species richness (i.e., total number), can be a useful criterion to describe the biological quality of a stream reach (Karr 1981). Low species richness values may indicate that a stream has been subject to one or more perturbations (e.g., pollution), while high values suggest a more stable or quality environment. Species richness of each sample was determined from raw and gear efficiency adjusted data.

Diversity is a measure of how the number of individuals are divided among the species in a community and it can be useful to describe the structure of a fish community. Maximum diversity of a community is when the individuals are distributed as evenly as possible among species (Pielou 1966), which suggest a more stable or quality environment. Shannon-Weaver diversity indices in (Pielou 1966) were calculated from raw and gear efficiency adjusted data.

The index of biotic integrity (IBI) is commonly used as an indicator of stream quality (Karr 1981, Fausch et al. 1984) and

Table 1. Coefficients and extra-binomial variance (σ^2) for backpack electrofisher and 2 run efficiency model from Bayley and Dowling (1990). See Table 3 for species group membership.

Species group	Variable	Coefficient	σ^2
PIK	Constant	-3.82	1.49
	Fish length (cm)	0.112	
OPN	Constant	-2.35	0.981
MNO	Constant	-0.759	0.116
	Fish length (cm)	0.316	
	Mean velocity (ft/s)	-2.89	
	Conductivity (μohms)	-0.00487	
	Physical impedance	0.633	
SUC	Constant	-3.40	0.208
	Fish length (cm)	0.0648	
	Physical impedance	0.910	
CAT (PIN)	Constant	-3.77	0.544
TOP	Constant	4.00	0.369
	Fish length (cm)	0.645	
	Conductivity (μohms)	-0.0144	
BAS	Constant	-2.10	0.663
SUN	Constant	-2.09	0.218
DAR	Constant	-9.71	0.000
	Mean velocity (ft/s)	-7.160	
	Physical impedance	-0.834	
	Temperature ©	0.289	
Species-richness	Constant	3.40	0.136
	Mean velocity (ft/s)	-1.95	
	Conductivity (μohms)	-0.00372	

Table 2. Coefficients and extra-binomial variance (σ^2) for 30 ft minnow seine 2 run efficiency model from Bayley and Dowling (1990). See Table 3 for species group membership.

Species group	Variable	Coefficient	σ^2
PIK	Constant	-1.640	0.269
	Fish length (cm)	0.092	
	Mean velocity (ft/s)	-1.14	
OPN	Constant	-2.49	0.00
MNO	Constant	-6.41	0.406
	Fish length (cm)	1.67	
	Fish length^2 (cm^2)	-0.199	
	Mean velocity (ft/s)	-0.304	
	Mean stream width (ft)	-0.0331	
	Mean depth (inches)	0.133	
SUC	Constant	-0.562	0.065
	Mean velocity (ft/s)	-3.47	
	Mean stream width	-0.0547	
CAT (PIN)	Constant	-4.07	7.70
	Physical impedance	-2.30	
TOP	Constant	1.48	0.111
	Fish length (cm)	-1.20	
	Physical impedance	-0.541	
	PAS	0.0196	
BAS	Constant	-3.16	0.000
	Fish length (cm)	0.425	
	Fish length^2 (cm^2)	0.425	
SUN	Constant	-11.7	0.824
	Fish length (cm)	2.78	
	Fish length^2 (cm^2)	-0.202	
DAR	Constant	-4.56	0.670
	Fish length (cm)	1.01	
	Mean velocity (ft/s)	-2.95	
	Stream width (ft)	-0.133	
	% riffle	-0.0417	
Species-richness	Constant	1.620	0.000
	Width	-0.0353	
	% riffle	-0.0398	

Table 3. Fish species collected during the study period. Bold face code represents species groups used for efficiency models. Asterisk represents species used during gear efficiency model verification. Species type for index of biotic integrity from Hoefs (1989); darter (DAR), sculpin (PIN), minnow (MNO), water column minnow (CMO), sunfish (SUN), and round bodied sucker (SUC). Ecological tolerance: intolerant species (I), tolerant species (T). Spawning guilds: nest builders (N), complex spawners with parental care (C), miscellaneous substrate (M), simple lithophilous (L), unknown (U), other (O). Trophic guild of adult fish: piscivore (P), invertivore/ piscivore (IP), omnivore (O), herbivore/detritivore (H), planktivore (Pl), unknown (U).

Common name	Scientific name	Type	Tolerance	Spawning	Trophic
PIK					
Grass pickerel	*Esox americanus*			M	P
Chain pickerel	*Esox niger*			M	P
MNO					
Hornyhead chub*	*Nocomis biguttatus*	MNO		N	I
Creek chub*	*Semotilus atromaculatus*	MNO	T	N	IP
Golden shiner*	*Notemigonus crysoleucas*	MNO	T	M	O
Red shiner*	*Cyprinella lutrensis*	MNO		M	I
Spotfin shiner*	*Cyprinella spiloptera*	CMO	I	M	W
Whitetail shiner	*Cyprinella galactura*	CMO	I	M	W
Striped shiner*	*Luxilus chrysocephalus*	MNO		S	I
Bleeding shiner*	*Luxilus zonatus*	CMO	I	N	W
Duskystripe shiner*	*Luxilus pilsbryi*	CMO	I	N	W
Redfin shiner*	*Lythrurus umbratilis*	CMO		M	I
Bigeye shiner*	*Notropis boops*	CMO	I	U	W
Wedgespot shiner	*Notropis greenei*	CMO	I	S	W
Ozark minnow*	*Notropis nubilus*	MNO	I	S	H
Rosyface shiner*	*Notropis rubellus*	CMO	I	S	W
Sand shiner*	*Notropis stramineus*	MNO	I	S	H
Telescope shiner	*Notropis telescopus*	CMO	I	U	W
Bluntnose minnow*	*Pimephales notatus*	MNO	T	C	O
Central stoneroller*	*Campostoma anomalum*	MNO		N	H
MNO					
Largescale stoneroller*	*Campostoma oligolepis*	MNO		N	H
Southern redbelly dace*	*Phoxinus erthrogaster*	MNO	I	S	H
SUC					
White sucker*	*Catostomus commersoni*		T	M	O
Creek chubsucker*	*Erimyzon oblongus*		I	M	I
Speckled chub	*Hybopsis aestivalis*	SUC		S	H
Northern hog sucker*	*Hypentelium nigricans*	SUC	I	S	B
Spotted sucker	*Minytrema melanops*	SUC	I	S	B
Black redhorse*	*Moxostoma duquesnei*	SUC	I	S	B
Golden redhorse*	*Moxostoma erythrurum*	SUC		S	B

Table 3 (continued).

Common name	Scientific name	Type	Tolerance	Spawning	Trophic
CAT					
Yellow bullhead*	*Ameiurus natalis*		T	C	O
Ozark madtom*	*Noturus albater*			C	B
Slender madtom*	*Noturus exilis*			C	B
Checkered madtom	*Noturus flavater*		I	C	B
Stonecat*	*Noturus flavus*			C	B
TOP					
Northern studfish*	*Fundulus catenatus*			S	I
Blackspotted topminnow*	*Fundulus olivaceus*			M	W
Plains topminnow	*Fundulus sciadicus*			M	W
Mosquitofish*	*Gambusia affinis*			O	I
Brook silverside	*Labidesthes sicculus*		I	M	W
Mottled sculpin*	*Cottus bairdi*	PIN	I	C	B
Banded sculpin*	*Cottus carolinae*	PIN		C	B
BAS					
Smallmouth bass*	*Micropterus dolomieu*			C	P
Spotted bass	*Micropterus punctulatus*			C	IP
Largemouth bass*	*Micropterus salmoides*			C	P
SUN					
Rock bass*	*Ambloplites rupestris*	SUN	I	C	IP
Green sunfish*	*Lepomis cyanellus*		T	C	I
Warmouth	*Lepomis gulosus*	SUN		C	I
Bluegill*	*Lepomis macrochirus*	SUN		C	I
Longear sunfish*	*Lepomis megalotis*	SUN	I	C	I
DAR					
Logperch*	*Percina caprodes*	DAR		S	B
Guilt darter	*Percina evides*	DAR	I	S	B
Greenside darter*	*Etheostoma blennioides*	DAR	I	M	B
Rainbow darter*	*Etheostoma caeruleum*	DAR		S	B
Fantail darter*	*Etheostoma flabellare*	DAR		C	B
Yolk darter	*Etheostoma juliae*	DAR		U	B
Niangua darter	*Etheostoma nianguae*	DAR	I	M	B
Johnny darter*	*Etheostoma nigrum*	DAR		C	B
Stippled darter	*Etheostoma punctulatum*	DAR	I	U	U
Orangethroat darter*	*Etheostoma spectabile*	DAR		S	B
Missouri saddled darter	*Etheostoma tetrazonum*	DAR	I	M	B
Banded darter*	Etheostoma zonale	DAR	I	M	B
OPN					
Rainbow trout	*Oncorhynchus mykiss*			S	IP
Freshwater drum	*Aplodinotus grunniens*			M	IP

has five stream quality classes with scores that range from 10-very poor, to 50–excellent. The IBI is a region specific combination of several community attributes that provide information about the structural and functional components of the resident fish community. IBI scores were obtained by summing community indices developed for Ozark stream fish communities (Hoefs 1989; Table 4), using gear efficiency adjusted data.

Stream type (i.e., impacted or unimpacted) were identified by examining biotic indices from a preliminary analysis of aquatic invertebrate data.

Within site variation of community indices was assessed with a mixed model (model III) ANOVA using stream type (i.e., impacted vs. unimpacted) as a fixed factor and reach as a random factor. A mixed model ANOVA differs from the more familiar fixed (model I) and random (model II) models by containing both fixed and random factors (Neter et al. 1990), the designation of which depends mainly upon intent of the analysis (Lewis 1978) provided that the assumptions regarding proper randomization are fulfilled.

A crossed nested design ANOVA was used to test the significance of the differences in community index scores between stream types and to assess the variability among reaches within a stream. With this design, the variability among reaches could be assessed without being affected by differences between stream types. Residuals were inspected for normality, constancy of variance, and independence.

Within site variance (i.e., among reaches) was estimated from the ANOVA expected mean squares following Snedecor and Cochran (1967):

$$S2R = (MSR - MSE)/n$$

where: S2 = variance, MS = mean square, R = reaches, E = error, and n = the harmonic mean of the number of reaches sampled at each site.

To determine the number of samples needed to detect changes in fish community indices, we assumed that a t-test would be used to compare indices from one year to the next or before and after implementation of a new management strategy. Thus, the required sample size was calculated by using a rearrangement of the t-test formula (Parkinson et al. 1988):

$$N = 100^2 k(SD/X)^2/p^2$$

where N is the required number of samples (i.e., reaches), k is a constant that varies with α level and statistical power (Snedecor and Cochran 1967), SD and X are the standard deviation of the among reach variance (i.e., square root of S2R) and community index mean for unimpacted streams respectively, and p is the percent detectible change. Graphs of N versus p were generated with $\alpha = 0.05$, 90% statistical power, and assuming a one-tailed test (i.e., $k = 17.13$).

RESULTS

Gear Evaluation

Eleven gear efficiency model verifications, five minnow seine and six backpack electrofisher, were conducted during late summer-early fall 1995. Verifications covered a wide range of physical and chemical conditions (Table 5) and included 44 species representing seven efficiency groups (Table 3). Pickerel (*Esox* spp.) and freshwater drum were uncommon at most study sites. Consequently, we were unable to verify efficiency models for the PIK and OPN groups. In addition, at some sites very few individuals of some species groups were collected and marked, which resulted in several zero measured efficiencies for groups that had less than three marked individuals. Therefore, we only included measured efficiencies for cases where more than three fishes were marked.

Twelve of 14 or 85.7% of the measured efficiencies for the backpack electrofisher were within the predicted 95% confidence intervals (Fig. 1a). Measured efficiencies outside the 95% confidence intervals, a SUC and MNO group, were both slightly lower than predicted efficiencies. In

Table 4. Metrics and scoring criteria modified to assess fish communities in Missouri streams from Hoefs (1989).

Category	Metric	Scoring criteria 5	3	1
Species richness and composition	1. Total number of native species	>9	4-9	<4
	2. Number and identity of darter, sculpin and round bodied sucker species	>3	2-3	<2
	3. Number and identity of sunfish and water column minnow species	>3	0-3	0
	4. Number and identity of sucker, minnow, and species water column minnow species	>5	3-5	<3
	5. Number and identity of intolerant species	>2	2	<2
	6. Proportion of individuals as green sunfish	<5%	5-20%	>20%
	7. Proportion of individuals as omnivores	<20%	45-20%	>45%
	8. Proportion of individuals as insectivorous minnows	>45%	45-20%	<20%
	9. Proportion of individuals as piscivores	>15%	<15%	
	10. Proportion of individuals as lithophilic spawners	>45%	45-20%	<20%

Table 5. The means, ranges, and standard errors (SE) of physical habitat characteristics for 11 DC backpack electrofisher and minnow seine efficiency verifications.

Habitat characteristic	Mean	Range	SE
Length (ft)	182.5	100-390	28.0
Width (ft)	10.3	6-16	0.88
Depth (in)	13.2	7-22	1.50
Velocity (ft/s)	0.48	0.08-1.03	0.08
Conductivity (µohms)	409.5	315-650	28.5
Temperature ©	18.5	16-22	0.78
Physical impedance (0-3)	1.25	0-3	0.17
% vegetation	2.68	0-10	2.68
% riffle	14.1	9-25	1.82

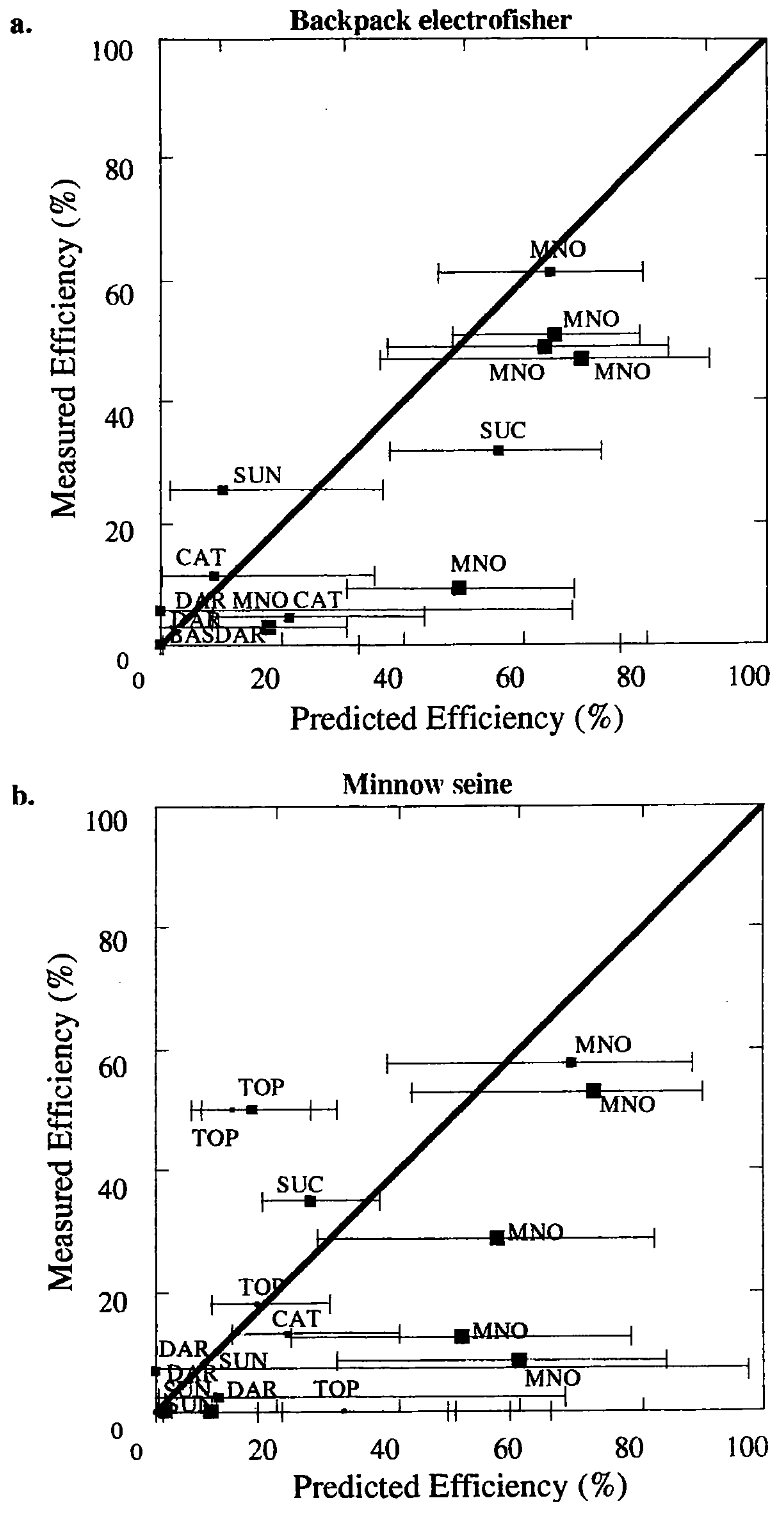


Fig. 1. Measured vs. predicted efficiency, with 95% confidence intervals, of 1) backpack electrofisher and b) minnow seine for species groups in Missouri Streams. Measured efficiency based on recapture of marked fish. Predicted efficiency from Bayley and Dowling (1990) models. See Table 3 for species group designations. Size of points proportional to number of fish marked.

addition, efficiencies of the backpack electrofisher were greater than the minnow seine for most species groups (Fig. 1a and b).

Predicted minnow seine efficiencies were fairly accurate with 82.4% of measured efficiencies within the predicted 95% confidence intervals (Fig. 1b). Of the species groups outside the 95% confidence intervals, measured efficiencies were greater than predicted for two of the three cases (Fig. 1b).

A comparison of raw (i.e., unadjusted) backpack electrofisher and minnow seine catches at the same site suggested differences in gear efficiencies. Raw species richness of 72% of the gear evaluations was much lower for the minnow seine and, in many cases, minnow seine estimates were more than 30% less than the backpack electrofisher (Fig. 2a). Similar to species richness, raw community diversity of all minnow seine catches were markedly lower than the backpack electrofisher (Fig. 2c). After gear efficiency adjustments, richness and diversity estimates of both gear overlapped considerably (Fig. 2b and d). In addition, secondary gear estimates were not consistently higher or lower than primary gear estimates, suggesting that the use of a primary gear did not influence efficiency of the secondary gear (Fig. 2a-d).

Community Indices

During late summer-early fall 1995, fish community structure was examined in 29 Missouri streams, 23 unimpacted and 6 impacted, to determine effects of stream quality. Fishes were sampled with both gear types resulting in 12 and 3 backpack electrofisher and 11 and 3 minnow seine samples from unimpacted and impacted streams, respectively.

Across gear, species richness did not differ significantly ($P = 0.96$) between impacted and unimpacted streams (Table 6). Gear specific species richness by stream type suggested a slightly greater richness at backpack electrofisher sites regardless of stream type (Fig. 3a). Nonetheless, the extensive overlap of 95% confidence intervals for both gear indicated no significant differences between unimpacted and impacted sites (Fig. 3a and b).

Similar to species richness, community diversity did not differ significantly ($P = 0.18$) between impacted and unimpacted streams (Table 7). Gear specific community diversity by stream type also suggested a slightly greater diversity at the backpack electrofisher sites regardless of stream type (Fig. 4a). In addition, overlap of 95% confidence intervals for both gear indicated no significant differences between unimpacted and impacted sites (Fig. 4a and b).

In contrast to richness and diversity, the IBI was significantly greater ($P = 0.02$) in unimpacted streams, across gear (Table 8). The IBI in unimpacted streams, average 38.2, was 13% greater than impacted streams and were classified as fair' according to Hoefs (1989). In contrast, gear specific IBI estimates indicated nonsignificant differences between impacted and unimpacted streams where the minnow seine was used, whereas streams that used the backpack electrofisher were significantly different (Fig. 5a and b).

Nonsignificant differences for species richness and community diversity between unimpacted and impacted streams suggested that these two indices may not be sensitive to stream quality impacts. Consequently, the number of samples needed to detect potential impacts was only determined for the IBI. The number of reaches that need to be sampled to detect changes in the IBI from 1 year to the next or detect differences between stream types was surprisingly high. For example, to detect a 13% decrease in the IBI of unimpacted streams, the average difference between impacted and unimpacted, seven to eight stream reaches need to be sampled (Fig. 6). In addition, the more than 13 reaches needed to be sampled to detect changes of less than 10% may be cost prohibitive.

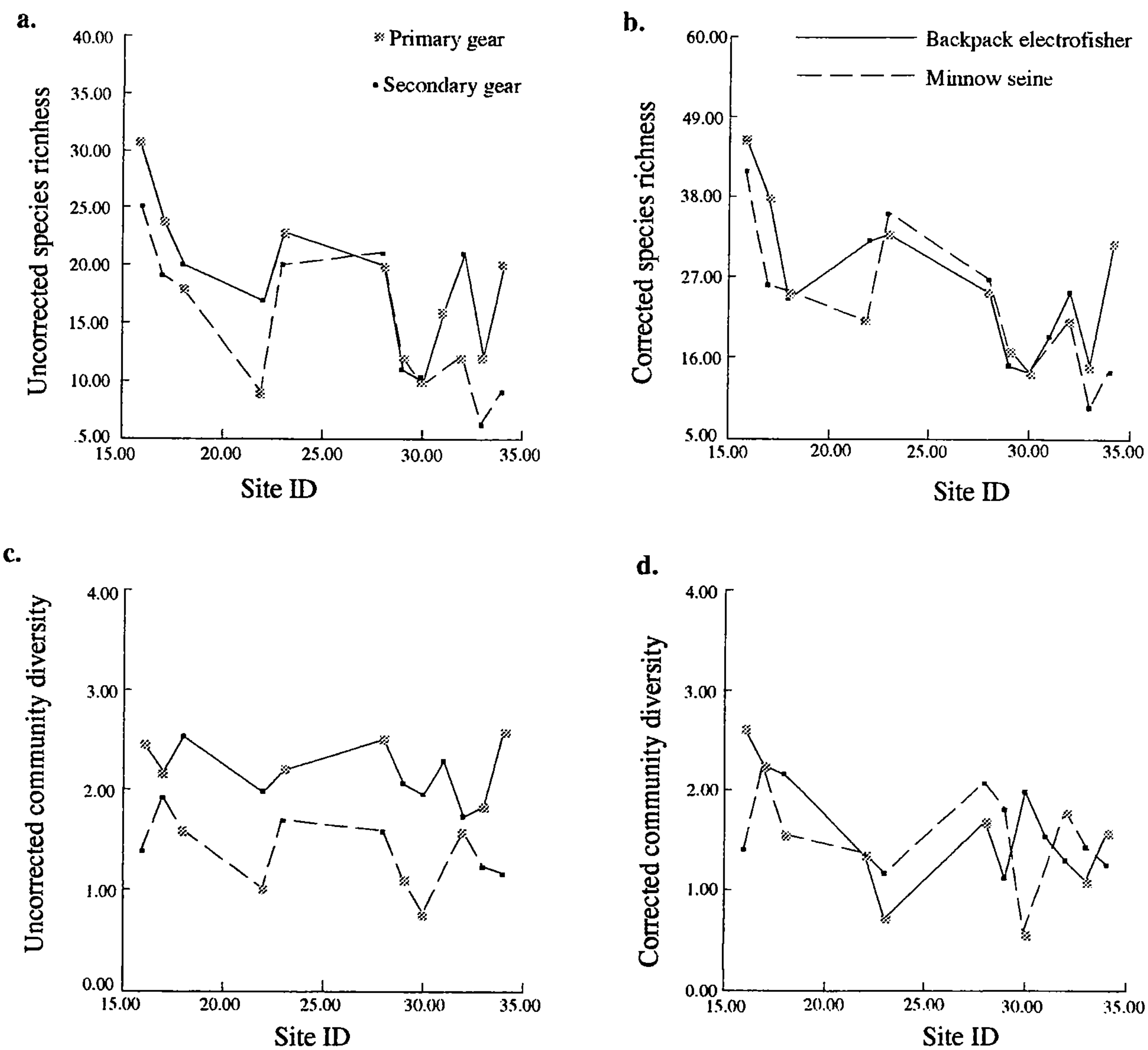


Fig. 2. Gear efficiency corrected and uncorrected species richness (a and b) and community diversity (c and d) by method at the 11 gear efficiency verification streams. Primary gear denoted by lightly shaded point and secondary gear by solid black point.

Table 6. Mixed model analysis of variance of fish species richness in impacted and unimpacted streams in Missouri; n = 56, r^2 = 0.245.

Source	df	Sum-of-squares	Mean-square	F-ratio	P-value
Stream type	1	0.1844	0.1844	0.0024	0.9610
Reach within stream type	7	1117.8769	159.6967	2.0894	0.0632
Error	47	3592.2231	76.4303		

Table 7. Mixed model analysis of variance of fish community diversity in impacted and unimpacted streams in Missouri; n = 56, r^2 = 0.163.

Source	df	Sum-of-squares	Mean-square	F-ratio	P-value
Stream type	1	0.4748	0.4748	1.8352	0.1820
Reach within stream type	7	1.9778	0.2825	1.0920	0.3837
Error	47	12.1610	0.2587		

Table 8. Mixed model analysis of variance of the index of biotic integrity for fish communities in impacted and unimpacted streams in Missouri; n = 56, r^2 = 0.163.

Source	df	Sum-of-squares	Mean-square	F-ratio	P-value
Stream type	1	149.1412	149.1412	5.5477	0.0227
Reach within stream type	7	376.2737	53.7533	1.9995	0.0736
Error	47	1263.5273	26.8836		

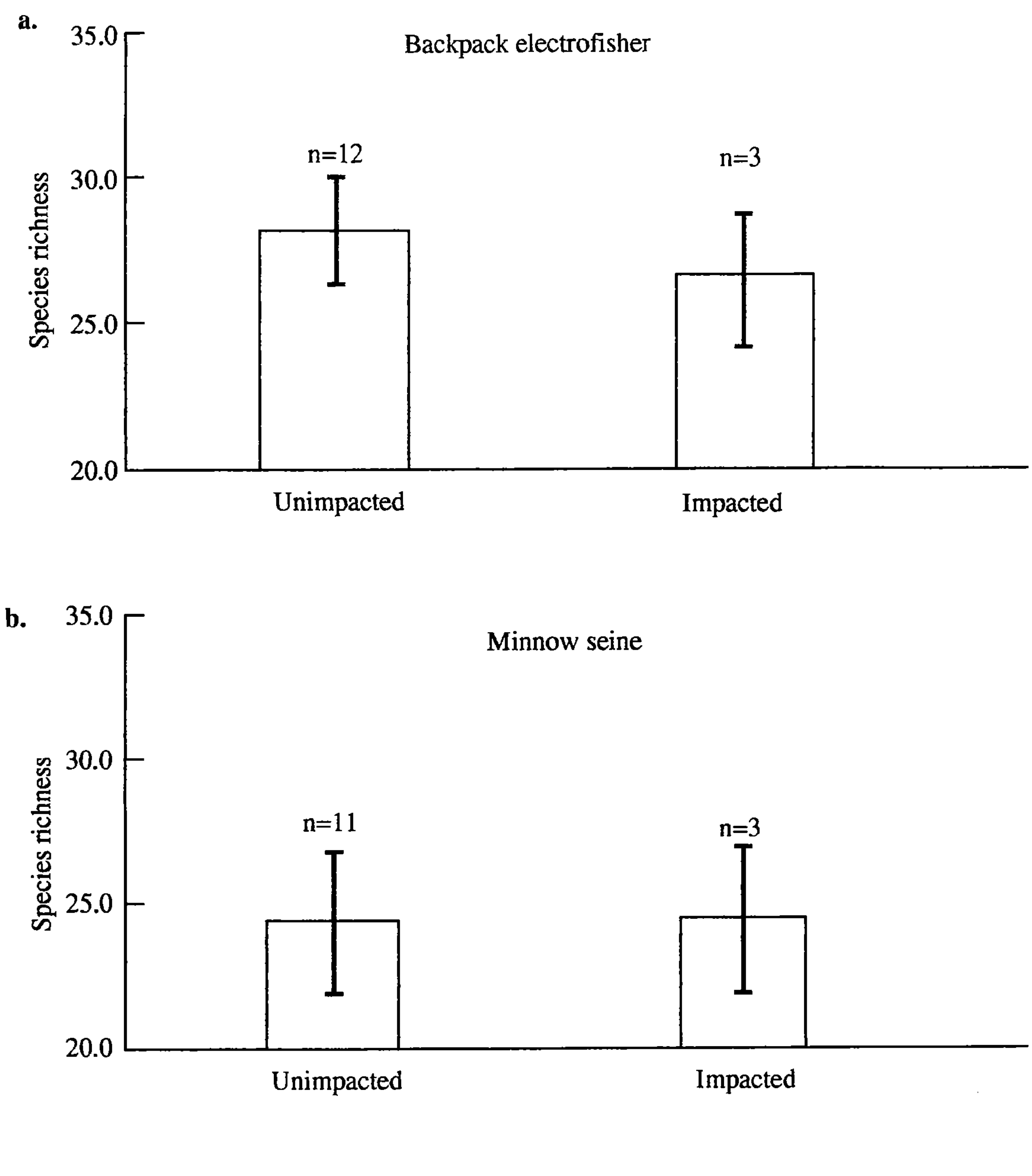
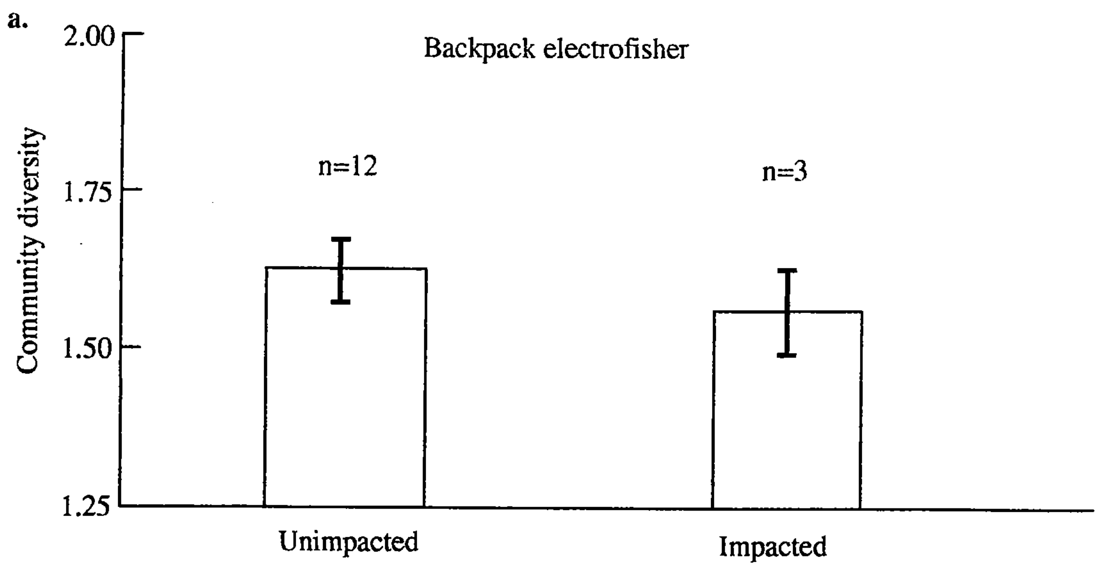


Fig. 3. Mean species richness, wit 95% confidence intervals, for fish communities in impacted and unimpacted Missouri streams, by fish collection method. Number of streams sampled are above bars.



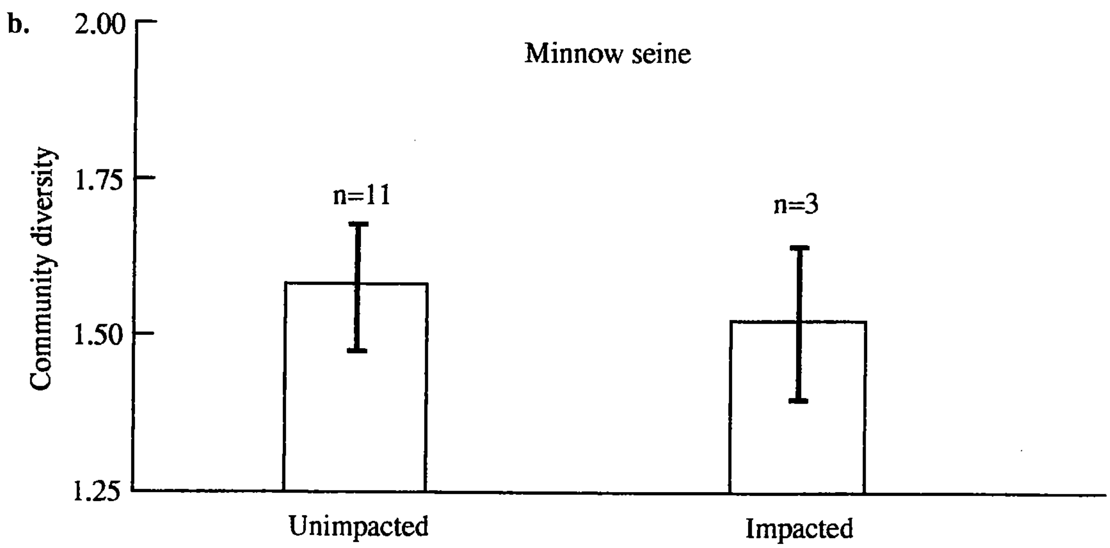


Fig. 4. Community diversity, with 95% confidence intervals, for fish communities in impacted and unimpacted Missouri streams, by fish collection method. Number of streams sampled are above bars.

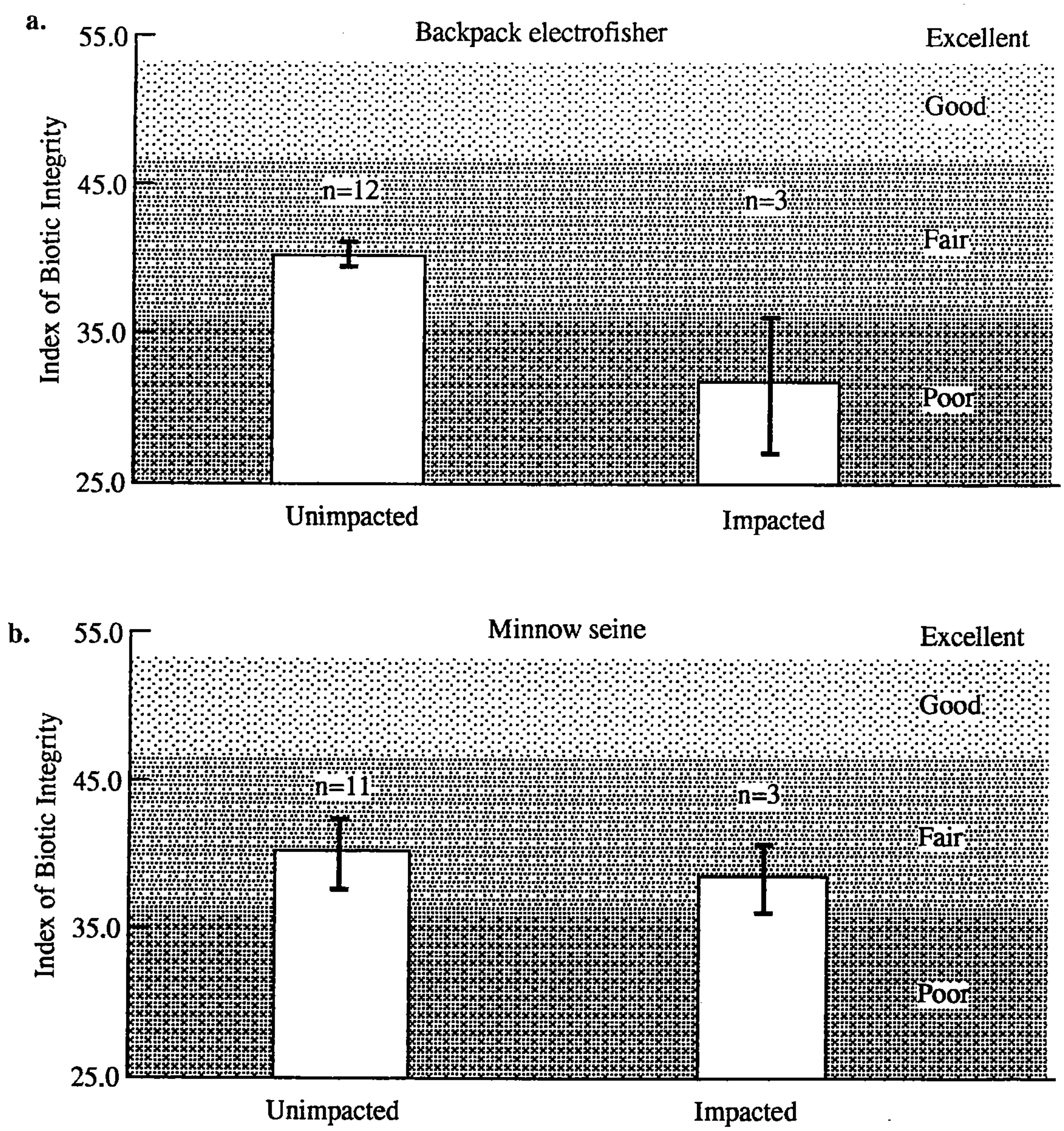


Fig. 5. Mean index of biotic integrity, with 95% confidence intervals, for fish communities in impacted and unimpacted Missouri streams, by fish collection method. Number of streams sampled are above bars. Shaded areas represent stream quality ratings as defined by Hoefs (1989).

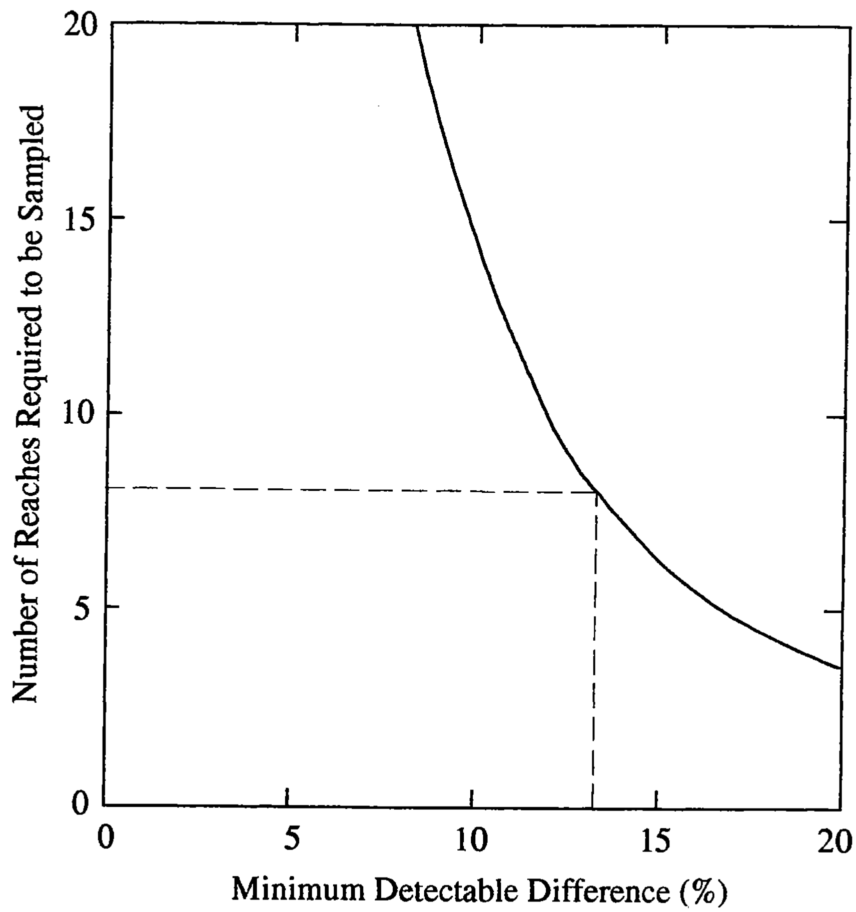


Fig. 6. Number of reaches needed to be sampled to detect various differences in the index of biologic integrity (IBI) with a one-tailed test at $\alpha = 0.05$ and 90% statistical power. Broken line indicates average difference (13%) between impacted and unimpacted IBI scores in Missouri streams and the corresponding number of reaches needed to detect the difference.

DISCUSSION

Gear Selection

The Bayley and Dowling (1990), BD, gear efficiency models were fairly accurate at predicting efficiencies for most species groups. Gear efficiency is affected by a combination of habitat characteristics (e.g., depth) and species specific traits (e.g., morphology; Bayley and Dowling 1990). Many of the streams in Missouri are very similar to stream reaches used for BD calibrations (pers. observation). Fish assemblages in the present study and the BD calibration were also similar, with almost 50% of species in common. Thus, the similarities in physical habitat and fish assemblages were probably responsible for the accuracy of the BD models. PIK and OPN efficiency models were not verified because of the inability to collect and mark species in these groups. Chain and grass pickerel (PIK) and freshwater drum (OPN) use habitats similar to conspecifics in different systems (Pflieger 1975), and the latter two species were used to calibrate BD models (Bayley and Dowling 1990). Assuming that the relative accuracy of BD models was due to similarities in physical habitat and species assemblage (discussed above), the Bayley and Dowling PIK and OPN models should accurately predict actual PIK and OPN gear efficiencies. In addition, comparisons of raw and adjusted data suggest that raw catch data were, to some extent, biased (Fig. 2a-d). Consequently, we recommend use of efficiency model coefficients (Tables 1 and 2) to adjust the raw catch data for all species and species richness in Missouri streams, provided they are within the range of physical and chemical conditions under which the gear were calibrated (Table 9).

Low sampling efficiency can increase sample variance by increasing sampling error (Peterson and Rabeni 1995). Efficiency is in part influenced by gear type, which, in turn, can influence the magnitude of variation of fish community indices. The minnow seine is, in general, less efficient for most species groups under conditions encountered in Illinois and presumably similar Missouri streams (Bayley and Dowling 1990). Consequently, sample variance of minnow seine estimates were generally greater than backpack electrofisher (i.e., larger 95% confidence intervals [Figs. 3- 5]). High variance of an established sampling protocol can only be overcome by increasing sample size (Snedecor and Cochran 1967, Sokal and Rohlf 1981). Therefore, required sample sizes (i.e., number of reaches) needed to detect changes in stream quality would be larger for a sampling protocol that used a minnow seine rather than the backpack electrofisher, possibly increasing the overall cost of the protocol.

Gear type may also affect the value of a community index even if data are adjusted for gear efficiency. For instance, the IBI calculated with minnow seine data was not significantly different between unimpacted and impacted streams; whereas backpack electrofisher IBI data were different (Fig. 5a and b). The IBI uses the proportion of green sunfish as an indicator of stream health. The greater the proportion of sunfish, the lower the score. In general, the minnow seine is much less efficient at collecting green sunfish than the backpack electrofisher (Bayley and Dowling 1990). Although seine and electrofisher data were adjusted for efficiency, very low efficiencies may result in zero catches that cannot be adjusted (i.e., the adjustment for zero sunfish is still zero sunfish). Therefore, the proportion of green sunfish may have been underestimated in impacted streams sampled with the minnow seine, resulting in greater than expected IBI scores. Given this possible source of bias and effects of efficiency on variance (discussed above), we recommend that stream quality monitoring protocols use a backpack electrofisher to sample fishes in Missouri.

Index Selection

Sensitivity to environmental degradation is probably the most desirable

Table 9. The means and ranges of physical habitat characteristics measured during Bayley and Dowling (1990) backpack electrofisher and minnow seine efficiency calibrations.

Habitat characteristic	Backpack electrofisher		Minnow seine	
	Mean	Range	Mean	Range
Width (ft)	21.3	3.5-40	19.9	3-45
Depth (inches)	12.6	4-24	12.2	4-20
Velocity (ft/sec)	0.27	0.03-0.56	0.26	0-0.68
Conductivity (μohms)	610	485-750		
Temperature ©	20.5	17-26	19.5	11-27
Physical impedance (0-3)	0.62	0-3	1.0	0-3
% vegetation	24.2	0-95	6.3	0-45
% riffle	5.62	0-20	4.92	0-35

property of a stream quality index. We found no significant differences in species richness and community diversity between impacted and unimpacted streams, whereas the IBI did detect differences. This is consistent with previous investigations of fish community structure and environmental degradation (reviewed in Fausch et al. 1990). The relative insensitivity of species richness and diversity are probably due to their inability to account for species identity. In many stream systems, there is a continual replacement of species from the headwaters to downstream (Vannote et al. 1980), so that the identity of resident species may differ among reaches. Yet, the total number of species or community diversity may remain constant. In contrast, the IBI takes into account species specific properties, such as tolerance and intolerance to environmental degradation, and is more sensitive to changes in stream quality (Karr 1981, Fausch et al. 1990). Therefore, we recommend that water quality monitoring protocols use the regional specific IBI (Hoefs 1989) to establish baseline stream conditions and to detect changes in the quality of Missouri streams.

Sampling Protocol

An optimal sampling protocol takes into account the cost of collecting samples in space and time and attempts to minimize both variance and costs. Peterson and Rabeni (1995) suggested that fish samples be collected during a single late summer period to minimize variance and costs, but indicated that required sample sizes should be determined for individual studies. We found that a minimum of seven reaches (i.e., pool-riffle sequences) need to be sampled to detect a change in quality from fair to poor in Missouri streams. In streams without well defined pool-riffle patterns we recommend that the site be seven reaches (6 stream widths per reach, or a total of 42 times the mean stream width) to maintain a certain amount of consistency between different streams. The number of reaches required assumed a one-tailed test (i.e., test for either a decrease or increase in the IBI), and we caution that a two-tailed test would require additional stream reaches (Parkinson et al. 1988). In summary, we recommend that stream quality monitoring projects in low order Missouri streams sample fishes in a minimum of seven reaches with a backpack electrofisher, adjust data for gear efficiency, and use the regional IBI to determine the current status of streams and detect potential impacts on stream quality.

LITERATURE CITED

Allen, R. K. and G. F. Edmunds Jr. 1963a. A revision of the genus *Ephemerella* (Ephemeroptera:Ephemerellidae). VI. The subgenus *Serratella* in North America. Annals of the Entomological Society of America 56:583-600.

Allen, R. K. and G. F. Edmunds Jr. 1963b. A revision of the genus *Ephemerella* (Ephemeroptera:Ephemerellidae). VII. The subgenus *Eurylophella*. Canadian Entomology 95:597-623.

Allen, R. K. and G. F. Edmunds Jr. 1965. A revision of the genus *Ephemerella* (Ephemeroptera:Ephemerellidae). VIII. The subgenus *Ephemerella* in North America. Miscellaneous Publication of the Entomological Society of America 4:243-282.

Bagenal, T. B. 1979. EIFAC fishing gear intercalibration experiments. EIFAC Tech. Pap. 34. United Nations Food and Agriculture Organization, Rome.

Bailey, R. G. 1976. Ecoregions of the United States. Map (scale 1:7,500,000). USDA Forest Service, Intermountain Region, Ogden, UT.

Ball, J. 1982. Stream Classification Guidelines for Wisconsin. Wisconsin Department of Natural Resources Technical Bulletin, *In* Water quality standards handbook. 1983. U.S. EPA, Office of Water Regulations and Standards, Washington, DC.

Barbour, M. T., J. Gerrritsen and J. S. White. 1996. Development of the Stream Condition Index (SCI) for Florida. Tetra Tech Inc., Owings Mills. MD.

Barbour, M. T., J. L. Plafkin, B. P. Bradley, C. G. Graves and R. W. Wisseman. 1992. Evaluation of EPA's Rapid Bioassessment benthic metrics: metric redundancy and variability among reference stream sites. Environmental Toxicology and Chemistry 11:437-449.

Barbour, M. T. and J. B. Stribling. 1991. Use of habitat assessment in evaluating the biological integrity of stream communities. *In* U.S. EPA Biological criteria: research and regulation. EPA-440/5-91-005, Washington, DC.

Bartholow, J. M. 1989. Stream temperature Investigations: field and analytical methods. Instream flow information paper 13. Biological Report 89(17). Fish and Wildlife Service, U.S. Department of the Interior, Washington, DC.

Bauman, A. C. 1945. Report on a fisheries survey of the streams of Missouri with recommendations for their management. Missouri Conservation Commission, Jefferson City.

Bayley, P. B. and D. C. Dowling. 1990. Gear efficiency calibrations for stream and river sampling. Aquatic Ecology Technical Report 90/08. Illinois Natural History Survey, Champaign.

Bayley, P. B. and D. C. Dowling. 1993. The effects of habitat in biasing fish abundance and species richness estimates when using various sampling methods in streams. Pol. Arc. Hydrobiol. 40:5-14.

Bayley, P. B., R. W. Larimore and D. C. Dowling. 1989. The electric seine as a fish sampling gear in streams. Transactions of the American Fisheries Society 118:447-453.

Bednarik, A. F. and W. P. McCafferty. 1979. Biosystematic revision of the genus *Stenonema* (Ephemeroptera:Hepatageniidae). Canadian Bulletin of Fisheries and Aquatic Sciences 201:1-72.

Bode, R. 1988. Methods for rapid bioassessment of streams. Technical Report, New York State Environmental Conservation. 27pp.

Brown, A. V. and P. P. Brussock. 1991. Comparisons of benthic invertebrates between riffles and pools. Hydrobiologia 220:99-108.

Buchanan, T. J. and W. P. Sommers. 1969. Discharge measurements at gaging stations. Techniques of water-resources investigators of the U.S. Geological Survey, Book 3, Chapter A8. U.S. Geological Survey, Washington, DC. 65pp.

Caton, L. W. 1991. Improved subsampling methods for the EPA "Rapid bioassessment" benthic protocols. Bulletin of the North American Benthological Society 8:317-319.

Corkum, L. D. 1989. Patterns of benthic invertebrate assemblages in rivers of northwestern North America. Freshwater Biology 21:191-205.

Courtemanch, D. L. and S. P. Davies. 1987. A coefficient of community loss to assess detrimental change in aquatic communities. Water Research 21:217-222.

Crowley, J. M. 1967. Biogeography. Canadian Geography 11:312-326.

Crunkilton, R. L. and R. M. Duchrow. 1991. Use of stream order and biological indices to assess water quality in the Osage and Black river basins of Missouri. Hydrobiologia 224:155-166.

Cummins, K. W. 1973. Trophic relations of aquatic insects. Annual Review of Entomology 18:183-206.

Cummins, K. W. and M. A. Wilzbach. 1985. Field procedure for the analysis of functional feeding groups of stream macroinvertebrates. Contribution 1611. Appalachian Environmental Laboratory, Univ. Maryland, Frostburg. 19pp.

Davis, W. S. 1995. Biological assessment and criteria: building on the past. Pages 15-29, *in* W. S. Davis and T. P. Simon (eds.), Biological assessment and criteria. Lewis Publishers, Boca Raton, FL, and London, UK.

Davis, W. S. and T. P. Simon (eds.). 1995. Biological assessment and criteria. Lewis Publishers, Boca Raton, FL, and London, UK.

deBlij, H. J. 1978. Pages 3-8 *in* Introduction. Regions and concepts. John Wiley and Sons, Inc. New York, NY.

Fausch, K. D., J. R. Karr and P. R. Yant. 1984. Regional application of an index of biotic integrity based on stream fish communities. Transactions of the American Fisheries Society 113:39-55.

Fausch, K. D., J. Lyons, J. R. Karr and P. L. Angermeier. 1990. Fish communities as indicators of environmental degradation. American Fisheries Society Symposium 8:123-144.

Frissell, C. A., W. J. Liss, C. E. Warren and M. D. Hurley. 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. Environmental Management 10:199-214.

Gallant, A. L., T. R. Whittier, D. P. Larsen, J. M. Omernik and R. M. Hughes. 1989. Regionalization as a tool for managing environmental resources. EPA/600/3-89/060. U.S. EPA, Washington, DC.

Gauch, H. G. 1982. Multivariate analysis in community ecology. Cambridge University Press. 298pp.

Gold, D. 1969. Statistical tests and substantive significance. American Sociologist 4:42-46.

Gordon, N. D., T. A. McMahon and B. L. Finlayson. 1992. Stream hydrology: an introduction for ecologists. Wiley and Sons Ltd., West Sussex, UK.

Hamilton, K. and E. P. Bergersen. 1984. Methods to estimate aquatic habitat variables. Environmental Evaluation Project N. DPTS-35-9. Bureau of Reclamation, Denver Federal Center, Denver, CO.

Harrel, R. C. and T. C. Dorris. 1968. Stream order, morphometry, physicochemical conditions and community structure of macroinvertebrates in an intermittent stream. American Midland Naturalist 80:220-250.

Hebrank, A. W. 1989. Geologic natural features classification system for Missouri. Natural Areas Journal 9(2):106-116.

Heiskary, S. A., C. B. Wilson and D. P. Larsen. 1987. Analysis of regional patterns in lake quality: using ecoregions for lake management in Minnesota. Lake and Reservoir Management 3:337-344.

Hilsenhoff, W. L. 1982. Using a biotic index to evaluate water quality in streams. Wisconsin Department of Natural Resources Technical Bulletin. No. 132. 24pp.

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomology 20:31-39.

Hoefs, N. J. 1989. Applying the Index of Biotic Integrity to resource inventory in the Current River basin, Missouri. MS Thesis, Colorado State University, Fort Collins.

Huggins, D. G. and M. F. Moffett. 1988. Proposed biotic and habitat indices for use in Kansas streams. Report No. 35, Kansas Biological Survey, Lawrence, KS. 128pp.

Hughes, R. M., D. P. Larsen and J. M. Omernik. 1986. Regional reference sites: a method for assessing stream pollution. Environmental Management 10(5):625-629.

Hughes, R. M., E. Rexstad and C. E. Bond. 1987. The relationship of aquatic ecoregions, river basins, and physiographic provinces to the ichthyogeographic regions of Oregon. Copeia 2:423-432.

Hynes, H. B. N. 1961. The invertebrate fauna of a Welsh mountain stream. Archiv fur Hydrobiologie 57:344-388.

Hynes, H. B. N. 1970. The ecology of running waters. Univ. Toronto Press, Ontario, Canada.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Kolkwitz, R. and M. Marsson. 1902. Principles for the bioassessment of waterbodies according to their flora and fauna. Mitt. Der Klg. Pruf. fur Wass. Abw. Berlin-Dahlem 1:33-72.

Kondratieff, B. C. and J. R. Voshell Jr. 1984. The North and Central American species of *Isonychia* (Ephemeroptera:Oligoneuriidae). Transactions of the American Entomological Society 110:129–244.

Kovach, W. L. 1993. MSVP—a multivariate statistical package for IBM-PC's. Version 2.1. Kovach Computing Services, Pentraeth, Wales, UK.

Lafferty, B. 1987. A procedure for evaluating buffer strips for stream temperature protection under the Forest Practices Act. Pages 70-77 *in* Managing Oregon's riparian zone for timber, fish and wildlife. Technical Bulletin No. 514. National Council for Air and Stream Improvement, New York, NY.

Larsen, D. P., J. M. Omernik, R. M. Hughes, C. M. Rohm, T. R. Whittier, A. J. Kinney, A. L. Gallant and D. R. Dudley. 1986. The correspondence between spatial patterns in fish assemblages in Ohio streams and aquatic ecoregions. Environmental Management 10:815-828.

Lenat, D. L. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. Journal of the North American Benthological Society 7:222-233.

Lenat, D. R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. Journal of the North American Benthological Society 12:279-290.

Lewis, W. M. J. 1978. Comparison of temporal and spatial variation in the zooplankton of a lake by means of variance components. Ecology 59:666-671.

Lyons, J. 1986. Capture efficiency of a beach seine for seven freshwater fishes in a north-temperate lake. North American Jornal of Fisheries Management 6:288-289.

Lyons, J. 1989. Correspondence between the distribution of fish assemblages in Wisconsin streams and Omernik's ecoregions. American Midland Naturalist 122:163-182.

Mason, W. T. Jr. and P. P. Yevich. 1967. The use of phloxine B and rose bengal stain to facilitate sorting benthic samples. Transactions of the American Microscopical Society 86:221-223.

McCafferty, W. P. 1981. A distinctive new species of *Stenoneme*. Proceedings of the Entomological Society of Washington 83:512-515.

Merritt, R. W. and K. W. Cummins (eds.). 1984. An introduction to the aquatic insects of North America. Second Edition. Kendall/Hunt Publ. Co., Dubuque, IA.

Minshall, G. W. 1984. Aquatic insect substratum relationships. Pages 358–400 *in* V. H. Resh and D. M. Rosenburg (eds.), The ecology of aquatic insects. Praeger Publ., New York, NY.

Minshall, G. W., R. C. Peterson and C. F. Nimz. 1985. Species richness in streams of different size from the same drainage basin. Am. Midl. Nat. 125:16–38.

Missouri Department of Natural Resources. 1986. Missouri Water Atlas. Division of Geology and Land Survey, Rolla. 100pp.

Neter, J., W. Wasserman and M. Kutner. 1990. Applied linear statistical models. R. D. Irwin Inc, Homewood IL.

Norris, R. H., E. P. McElravy and V. H. Resh. 1992. The sampling problem. Pages 282-306 *in* P. Calow and G. E. Petts (eds.), Rivers handbook, Volume I. Blackwell Scientific Publs., Oxford, UK.

Novak, M. A. and R. W. Bode. 1992. Percent model affinity: a new measure of macroinvertebrate community composition. Journal of the North American Benthological Society 11:80-85.

Nuzzo, R. 1986. Macroinvertebrate rapid assessment methodology. Massachusetts Division of Water Pollution Control.

Omernik, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118-125.

Omernik, J. M. 1995. Ecoregions: a spatial framework for environmental management. *In* W. S. Davis and T. P. Simon (eds.), Biological assessment and criteria: tools for water resource planning and decision making. CRC Press, Florida.

Omernik, J. M. and A. L. Gallant. 1987. Ecoregions of the south-central states. EPA-600-D-87-315. U.S. Environmental Protection Agency, Corvallis, OR.

Osborne, L. L. and E. E. Herricks. 1983. Streamflow and velocity as determinants of aquatic insect distribution and benthic community structure in Illinois. Report No. UILU–WRC-83-183. Water Res. Center, Univ. Illinois; Bureau of Reclamation, U.S. Department of the Interior, Washington, DC.

Oswood, M. E. and W. E Barber. 1982. Assessment of fish habitat in streams: goals, constraints, and a new technique. Fisheries 7(3):8-11.

Parkinson, E., J. Berkowitz and C. Bull. 1988. Sample size requirements for detecting changes in some fisheries statistics from small trout lakes. North American Journal of Fisheries Management 8:181-190.

Parsons, M. and R. H. Norris. 1996. The effect of habitat-specific sampling on biological assessment of water quality using a predictive model. Freshwater Biology 36:419-434.

Pennak, R. W. 1989. Freshwater invertebrates of the United States—Protozoa to Mollusca. Third Edition. John Wiley and Sons, Inc., New York, NY. 628pp.

Pescador, M. L. and L. Berner. 1981. The mayfly family Baetiscidae (Epemeroptera). Part II. Biosystematatics of the genus *Baetisca*. Transactions of the American Entomological Society 107:163-228.

Peterson, J. T. and C. F. Rabeni. 1995. Optimizing sampling effort for sampling warmwater stream fish communities. North American Journal of Fisheries Management 15:528-541.

Pflieger, W. L. 1989. Aquatic Community Classification System for Missouri. Aquatic Series Number 19. Missouri Dept. Conservation, Jefferson City.

Pflieger, W. L., M. A. Schene Jr. and P. S. Haverland. 1981. Techniques for the classification of stream habitats, with examples of their application in defining the stream habitats of Missouri. Missouri Dept. Conservation, Fish and Wildlife Research Center, Columbia.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. Journal of Theoretical Biology 13:131-144.

Pinkham, C. F. A. and J. B. Pearson. 1976. Applications of a new coefficient of similarity to pollution surveys. Journal of the Water Pollution Control Federation 48:717-723.

Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross and R. M. Hughes. 1989. Rapid Bioassessment Protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA/444/4-89-001, Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, DC.

Platts, W. S., W. F. Megahan and G. W. Minshall. 1983. Methods for evaluating stream riparian and biotic conditions. General Technical Report INT–138. Forest Service, U.S. Department of Agriculture, Ogden, UT.

Poulton, B. C. and K. W. Stewart. 1991. The stoneflies of the Ozark and Ouachita Mountains (Plecoptera). Memoirs of the American Entmological Society #38. 116pp.

Provonsha, A. V. 1992. A revision of the genus *Caenis* in North America (Ephemeroptera:Caenidae). Transactions of the American Entomological Society 116:801-884.

Rabeni, C. F. and G. W. Minshall. 1977. Factors affecting the microdistribution of stream benthic insects. Oikos 29:33-43.

Reynolds, J. B. 1983. Electrofishing. Pages 147-163 *in* L. A. Nielson and D. L. Johnson (eds.), Fisheries Techniques. Southern Printing Co. Blacksburg, VA.

Riley, S., R. Haedrich and R. Gibson. 1993. Negative bias in removal estimates of Atlantic salmon parr relative to stream size. Journal of Freshwater Ecology 8:97-101.

Rodgers, J. D., M. F. Solazzi, S. L. Johnson and M. A. Buckman. 1992. Comparison of three techniques to estimate juvenille coho salmon populations in small streams. North American Journal of Fisheries Management 12:79-86.

Rohm, C. M., J. W. Giese and C. C. Bennett. 1987. Evaluation of an aquatic ecoregion classification of streams in Arkansas. Journal of Freshwater Ecology 4:127–140.

Rosenberg, D. M. and V. H. Resh. 1993. Freshwater biomonitoring and benthic invertebrates. Chapman and Hall, New York.

Schmid, F. 1968. La Famille des Arctopsychidaes. Mem. Entomol. Soc. Que., No. 1, Quebec, Canada.

Schueler, T. R. 1987. Controlling urban runoff: a practical manual for planning and designing urban BMPs. Publ. No. 87703. Metropolitan Washington Council of Governments, Washington, DC.

Shackleford, B. 1988. Rapid bioassessment of lotic macroinvertebrate communities. Arkansas Department of Pollution Control and Ecology, Little Rock. 44pp.

Shannon, C. E. and W. W. Weaver. 1949. The mathematical theory of communication. University of Illinois Press, Urbana. 125pp.

Snedecor, W. S. and W. G. Cochran. 1967. Statistical methods, 6th Edition. Iowa State Univ. Press, Ames. 593pp.

Sokal, R. and F. Rohlf. 1981. Biometry. W.H. Freeman and Co., San Francisco, CA.

Standard Methods for the Examination of Water and Wastewater. 1980. Published by the American Health Association, American Water Works Association, and Water Pollution Control Federation, 15th edition. Washington, DC.

Stark, J. D. 1993. Performance of the macroinvertebrate community index: effects of sampling method, sample replication, water depth, current velocity, and substratum on index values. New Zealand Journal of Marine and Freshwater Research 27:463-478.

Thom, R. H. and J. H. Wilson. 1980. The natural divisions of Missouri. Transactions of the Missouri Academy of Science 14:9-23.

Toft, C. A. and P. J. Shea. 1983. Detecting community-wide patterns: estimating power strengthens statistical inference 122:618-625.

U.S. EPA. 1983. Technical support manual: waterbody surveys and assessments for conducting use attainability analyses. Office of Water Regulations and Standards, Washington, DC.

U.S. EPA. 1991. Report of the Ecoregions Subcommittee of the Ecological Processes and Effects Committee: Evaluation of the Ecoregion Concept. EPA-SAB-EPEC-91-003. U.S. Environmental Protection Agency, Washington, DC.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Washington, H. G. 1984. diversity, biotic and similarity indices. Water Research 18:653-694.

Whittaker, R. H. And C. W. Fairbanks. 1958. A study of the plankton copepod communities in the Columbia basin, southwestern Washington. Ecology 39:46-65.

Whittier, T. R., R. M. Hughes and D. P. Larsen. 1988. Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. Canadian Journal of Fisheries and Aquatic Sciences 45:1264-1278.

Whittier, T. R., D. P. Larsen, R. M. Hughes, C. M. Rohm, A. L. Gallant and J. M. Omernik. 1987. The Ohio stream regionalization project: a compendium of results. EPA-600-3-87-025. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.

Wiley, M. J., L. L. Osborne and R. W. Larimore. 1990. Longitudinal structure of an agricultural prairie river system and its relationship to current stream ecosystem theory. Can. J. Fish. Aquat. Sci. 47:373-384.